

## BACLOFEN REDUCES POST-SYNAPTIC POTENTIALS OF RAT CORTICAL NEURONES BY AN ACTION OTHER THAN ITS HYPERPOLARIZING ACTION

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### SUMMARY

1. Intracellular recordings were obtained from neurones in layers 2 and 3 of the rat frontal neocortex in an *in vitro* slice preparation. Three distinct types of stimulation-evoked post-synaptic potentials were recorded in these neurones: excitatory post-synaptic potentials (e.p.s.p.s); bicuculline-sensitive, chloride-dependent inhibitory post-synaptic potentials (i.p.s.p.s) with times to peak of 20–25 ms (fast(f)-i.p.s.p.s); bicuculline-insensitive, potassium-dependent i.p.s.p.s with times to peak of 150–250 ms (long(l)-i.p.s.p.s).

2. The effects of baclofen were investigated on seventy-one neurones. Baclofen was applied by iontophoresis or pressure ejection from micropipettes or was added to the superfusion medium.

3. Baclofen depressed stimulation-evoked e.p.s.p.s in fifty-seven of the sixty neurones tested. This effect was associated with an increase in the stimulation intensity required to produce a synaptically evoked action potential for thirty-nine of forty-four neurones.

4. Baclofen depressed f-i.p.s.p.s in thirty-seven of the thirty-nine neurones tested and l-i.p.s.p.s in each one of the seventeen neurones tested. Reversal potential values for each type of i.p.s.p. were not changed by baclofen and its depressions of each were independent of membrane potential ( $E_m$ ). Baclofen reduced the magnitude and the duration of the conductance increases that were associated with f- and l-i.p.s.p.s.

5. Baclofen hyperpolarized forty of seventy-one neurones and produced outward currents in three of four neurones recorded in voltage clamp at holding potentials between  $-55$  and  $-65$  mV. These actions were associated with 10–58% reductions of neuronal input resistance ( $R_N$ ) and 10–20% increases in neuronal input conductance ( $g_N$ ), respectively. Baclofen decreased the direct excitability of twenty-three of twenty-seven neurones tested. Determinations of the reversal potential for baclofen-induced changes of  $E_m$  indicate that baclofen increases the conductance of rat neocortical neurones to potassium ions.

6. The  $EC_{50}$  for each action of DL-baclofen was approximately  $1 \mu M$ . L-Baclofen was  $> 100$  times more potent than D-baclofen.

7. Concentrations of bicuculline that blocked f-i.p.s.p.s and responses to ionophoretically applied  $\gamma$ -aminobutyric acid (GABA) had no effect on the depressions of e.p.s.p.s or the hyperpolarizations and decreases in  $R_N$  that baclofen produced.

8. Baclofen did not reduce the duration of action potentials that were prolonged with intracellular injections of caesium ions or by superfusions with medium that contained 10 mM-tetraethylammonium (TEA).

9. Almost complete depressions of post-synaptic potentials were observed for some neurones on which baclofen produced no hyperpolarization or apparent decrease in  $R_N$ . Baclofen's depressions of post-synaptic potentials outlasted the hyperpolarizations and the decreases in  $R_N$  and direct excitability that baclofen produced, often by several minutes. Applications of baclofen that produced almost complete depressions of post-synaptic potentials did not significantly reduce depolarizations produced by L-glutamate, L-aspartate, *N*-methyl-D-aspartate (NMDA), or GABA. At the same time that baclofen decreased resting  $R_N$ , conductance increases during stimulation-evoked i.p.s.p.s were reduced by baclofen. These results indicate that baclofen depresses post-synaptic potentials evoked in rat neocortical neurones by an action that is additional to and separate from its action to increase post-synaptic potassium conductance.

#### INTRODUCTION

In 1973, Pierau & Zimmermann reported that the GABA analogue baclofen ( $\beta$ -chlorophenyl-GABA) depressed e.p.s.p.s evoked in cat motoneurones at doses that had no effect on i.p.s.p.s or on the passive electrical properties or direct excitability of these cells. They concluded that baclofen acted presynaptically to inhibit central excitatory neurotransmission, and subsequent electrophysiological studies supported this conclusion (Davidoff & Sears, 1974; Fox, Krnjevic, Morris, Puil & Werman, 1978; Lanthorn & Cotman, 1981; Ault & Nadler, 1982; Olpe, Baudry, Fagni & Lynch, 1982). There have also been reports, however, that baclofen depresses i.p.s.p.s (Fox *et al.* 1978; Misgeld, Klee & Zeise, 1982; Scholfield, 1983) and has a direct hyperpolarizing action on some mammalian central neurones (Misgeld *et al.* 1982; Newberry & Nicoll, 1984; Brady & Swann, 1984). This hyperpolarizing action has been shown to be secondary to an increase in a voltage-dependent potassium conductance (Gähwiler & Brown, 1985; Inoue, Matsuo & Ogata, 1985a; Newberry & Nicoll, 1985).

It is now established that there are two classes of GABA receptor, GABA<sub>A</sub> and GABA<sub>B</sub>, and that baclofen is a prototypical agonist at GABA<sub>B</sub> receptors (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnball, 1980; Hill & Bowery, 1981). Because of the high density of GABA<sub>B</sub> receptor binding sites in the mammalian cerebral cortex (Karbon, Duman & Enna, 1983; Gehlert, Yamamura & Wamsley, 1985) and the multiple actions of baclofen observed in previous electrophysiological studies, we undertook to determine the effects of baclofen on rat neocortical neurones in an *in vitro* slice preparation. Some of the results were published previously in abstract form (Howe, Sutor & Zieglgänsberger, 1985; Sutor, Howe & Zieglgänsberger, 1985).

#### METHODS

Adult Sprague-Dawley rats (120–160 g) were anaesthetized with ether and decapitated. Their brains were removed rapidly, placed in oxygenated superfusion medium (4 °C), and sectioned with a razor blade such that transverse slices of each cerebral hemisphere could be cut in a plane of section

perpendicular to the dorsal surface of the frontal cerebral cortex. Eight serial slices (nominally 500  $\mu\text{m}$  thick) were cut from the frontal poles of each hemisphere with a McIlwain Tissue Chopper. After incubation at ambient room temperature for at least 1 h, slices (commonly those from the centre of each set) were placed in a continuous superfusion chamber and the temperature of the superfusion medium in this chamber was slowly heated to and maintained at 36–37 °C. The slices lay on a net and were superfused from below. Warm humidified air was circulated over the chamber to prevent the upper surfaces of the slices from drying.

The standard superfusion medium had the following composition (in mM): NaCl, 122.75; KCl, 5.00;  $\text{NaH}_2\text{PO}_4$ , 1.25;  $\text{CaCl}_2$ , 2.50;  $\text{MgSO}_4$ , 1.30;  $\text{NaHCO}_3$ , 26; glucose, 10. The superfusion medium was gassed continuously with 95 %  $\text{O}_2$ –5 %  $\text{CO}_2$  and had a final pH of 7.4. When the concentration of KCl in the standard medium was reduced, this was compensated for with equimolar NaCl. The medium in which TEA was applied contained (in mM): NaCl, 117.65; KCl, 2.50;  $\text{CaCl}_2$ , 2.50;  $\text{MgCl}_2$ , 1.30;  $\text{NaHCO}_3$ , 26; glucose, 10; TEA Cl, 10.

Solutions for superfusion were changed by switching a 4-way valve. The volume of the inflow system from this valve to the chamber was 0.8 ml and the chamber volume was 1.0 ml. Solutions were superfused at rates of 3.5–5 ml/min. The minimum time for replacement of the chamber volume with a new solution was therefore 20–30 s. With this superfusion system, responses to changes in the ionic composition of the medium required 5–10 min to reach steady state. All solutions were pre-warmed and equilibrated with 95 %  $\text{O}_2$ –5 %  $\text{CO}_2$ .

Intracellular recordings were obtained from neurones in cortical layers 2 or 3 of the dorsomedial region of the slices with glass micro-electrodes that were filled with either potassium acetate (4 M, pH adjusted to 7.0 with acetic acid), potassium methylsulphate ( $\text{KMeSO}_4$ , 2 M), or 1 M-CsCl. Electrodes typically had resistances of 50–80 M $\Omega$ . Post-synaptic potentials were evoked by electrical stimulation (0.05 ms in duration) of superficial cortical layers with a bipolar silver stimulation electrode that was insulated to within 250  $\mu\text{m}$  of the electrode tips (tip diameter: 100  $\mu\text{m}$ ; separation of tips: 250  $\mu\text{m}$ ). The stimulation electrode was positioned such that a line drawn between the tips would be approximately perpendicular to the dorsal surface of the cortex and so that the most dorsal tip of the bipolar electrode was 100–150  $\mu\text{m}$  from this surface. The stimulation electrode was placed 0.8–1.5 mm from the medial border of the slice and the tips were advanced into the slice 200–250  $\mu\text{m}$ . All recordings were obtained medial to and 0.4–1.0 mm away from the stimulation electrode. Conventional display techniques were used and signals were stored on magnetic tape (frequency response of tape recorder: 1.25 kHz). In some cases, the replayed signals were digitized and analysed with an LSI 11/73 computer.

Intracellular recordings and current injections were performed with a Dagan 8100 amplifier that was equipped with a bridge circuit and also with sample and hold single-electrode current-clamp and voltage-clamp modes (Wilson & Goldner, 1975; switching frequency set between 1 and 2 kHz, 25 % duty cycle). Some voltage-clamp recordings were made with a npi SEC1L (Fa. Polder, F.R.G.) single-electrode voltage-clamp amplifier (switching frequency, 10–12 kHz; duty cycle, 25 %). Determinations of  $R_N$ , neuronal input resistance, were made with 150 ms rectangular-current injections that produced hyperpolarizing voltage transients of < 10 mV or from the slope of current–voltage relation plots. When recordings were made in voltage clamp, neuronal input conductance,  $g_N$ , was estimated from the steady-state current responses to 150 ms, 10 mV hyperpolarizing voltage command steps. Membrane potential values were corrected for offset potentials that were determined by rapidly withdrawing the electrode at the end of the recording.

Baclofen was applied at known concentration in the superfusion medium or from micropipettes by ionophoresis or pressure ejection with a Neuro Phore  $\text{BH}_2$  unit. Unless it is stated otherwise, the racemic mixture was applied. Bicuculline methiodide was applied in the superfusion medium. L-glutamate, L-aspartate, *N*-methyl-D-aspartate (NMDA), and GABA were applied from micropipettes by ionophoresis. The 4-barrelled micropipettes (total tip broken to 3–5  $\mu\text{m}$ ) for ionophoresis or pressure ejection were positioned under microscopic control as close to the recording electrode as possible with an independent micromanipulator, advanced into the slice, and positioned to obtain maximal responses to ionophoretic ejections of L-glutamate and GABA. Solutions for ionophoresis were: NaCl, 1 M; baclofen, 10 mM, pH 4.0; GABA, 0.5 M; pH 4.5; sodium L-glutamate or sodium L-aspartate, 1 M, pH 8.0; NMDA, 25 mM; pH 8.0. Baclofen and GABA were ejected with cationic currents and L-glutamate, L-aspartate, and NMDA were ejected with anionic currents. Retaining currents (5–10 nA) were applied when the substances were not ejected actively. Automatic current neutralization was employed at all times.

In most cases, the range and mean  $\pm$  s.d. of the measured values are reported. In cases where the sample size is small or the sample values were not normally distributed, the median value is reported. Two-tailed Student's *t* tests were used for comparisons of two sample means. Quoted *n* values refer to the number of neurones for which the result was demonstrated. The reversibility of baclofen's effects is stated as full or partial, respectively, when the measured parameter recovered to within 75–100 % or 50–75 % of its value before baclofen.

## RESULTS

### *Characteristics of rat neocortical neurones*

Stable intracellular recordings of 1–6 h in duration were obtained from seventy-one neurones upon which baclofen was applied by iontophoresis (*n* = 15) or pressure ejection (*n* = 4) from micropipettes or by addition to the superfusion medium (*n* = 52). Some of the characteristics of these neurones and of their responses to current injections or to electrical stimulation of superficial cortical layers are summarized in Table 1. The current–voltage relations determined for these neurones were always non-linear and exhibited depolarizing and hyperpolarizing inward rectification. The dependence of  $R_N$  on the membrane potential,  $E_m$ , was further investigated in nineteen neurones by applying 150 ms current steps that produced small hyperpolarizing voltage transients after  $E_m$  was shifted to several values with longer current injections (> 800 ms). Neuronal input resistance was an increasing linear function of  $E_m$  between values of –100 to –60 mV. Linear regression analyses were performed on the data obtained over this  $E_m$  range and a rectification ratio,  $R_N$  at –65 mV/ $R_N$  at –95 mV, calculated from these analyses. On average,  $R_N$  determined at –65 mV was twice that determined at –95 mV (Table 1). At  $E_m$  values higher than –100 mV, the slope of  $R_N$  vs.  $E_m$  plots decreased smoothly toward zero. Neuronal input resistance was maximal between  $E_m$  values of –65 to –55 mV and usually decreased as  $E_m$  was depolarized beyond –55 mV.

Stimulation-evoked potentials were recruited in the following characteristic sequence as the stimulation intensity was increased: e.p.s.p.s with times to peak of 7–10 ms; i.p.s.p.s with times to peak of 20–25 ms; action potentials (Table 1). Several lines of evidence indicate that these fast i.p.s.p.s (f-i.p.s.p.s, times to peak of 20–25 ms) are secondary to GABA<sub>A</sub>-receptor-mediated increases in post-synaptic chloride conductance (Sutor & Zieglgänsberger, 1984). Because of the high resting  $E_m$  values of rat neocortical neurones *in vitro*, f-i.p.s.p.s were commonly depolarizing when evoked at resting  $E_m$  (Table 1; see also Connors, Gutnick & Prince, 1982). Whether or not depolarizing f-i.p.s.p.s were evoked with a given stimulation intensity could be determined by displacing  $E_m$  with current injections. The post-stimulus latency to onset of these f-i.p.s.p.s was obscured, however, by the preceding e.p.s.p.s. The amplitudes of depolarizing potentials evoked with stimulation intensities that evoked both e.p.s.p.s and f-i.p.s.p.s are therefore referred to as e.p.s.p.–i.p.s.p. amplitudes, although the amplitudes of these potentials were measured at their peaks 7–10 ms post-stimulus.

In many neurones, stimulation of superficial cortical layers also evoked i.p.s.p.s with times to peak of 150–250 ms and durations as great as 900 ms. These long i.p.s.p.s (l-i.p.s.p.s) can also be distinguished from preceding GABA<sub>A</sub>-receptor-mediated f-i.p.s.p.s on the basis of their reversal potentials ( $E_{i.p.s.p.}$ ), the dependence

TABLE 1. Electrophysiological properties of rat neocortical neurones upon which the effects of baclofen were evaluated. The range and the mean  $\pm$  standard deviation of each parameter and the number of neurones for which it was evaluated ( $n$ ) are presented. Abbreviations are defined in the text

	Range	Mean $\pm$ s.d.	$n$
Resting membrane potential	-56 to -95 mV	-80.4 $\pm$ 8.3 mV	71
Neuronal input resistance*	10 to 54 M $\Omega$	21.6 $\pm$ 10 M $\Omega$	64
Rectification ratio†	1.2 to 5.4	2.22 $\pm$ 0.90	19
Action potentials*‡			
Amplitude	84 to 134 mV	110 $\pm$ 11 mV	69
Overshoot	15 to 45 mV	30 $\pm$ 6.8 mV	69
Voltage threshold	-41 to -56 mV	-48 $\pm$ 4.8 mV	35
Post-synaptic potentials§			
e.p.s.p.s threshold	0.33 to 0.72	0.55 $\pm$ 0.09	60
f.i.p.s.p.s threshold	0.55 to 1.00	0.79 $\pm$ 0.09	36
$E_{i.p.s.p.}$ , 5.00 mM-K <sup>+</sup>	-65 to -71 mV	-68.7 $\pm$ 1.9 mV	11
$E_{i.p.s.p.}$ , 2.50 mM-K <sup>+</sup>	-66 to -74 mV	-71.3 $\pm$ 2.5 mV	18
l-i.p.s.p.s threshold	0.67 to 1.00	0.86 $\pm$ 0.07	17
$E_{i.p.s.p.}$ , 5.00 mM-K <sup>+</sup>	-79 to -86 mV	-81.4 $\pm$ 2.8 mV	6
$E_{i.p.s.p.}$ , 2.50 mM-K <sup>+</sup>	-84 to -97 mV	-89.7 $\pm$ 3.5 mV	11

\* Values determined for neurones that were recorded with CsCl-filled electrodes are not included.

† Ratio of  $R_N$  at  $E_m = -65$  mV to  $R_N$  at  $E_m = -95$  mV, which was determined as described in the text.

‡ Amplitudes and overshoots are for synaptically evoked action potentials and were measured from resting  $E_m$  to peak and from  $E_m = 0$  mV to peak, respectively. Voltage threshold refers to the  $E_m$  value (referenced to  $E_m = 0$  mV) at which action potentials were evoked by direct intracellular current injections.

§ Threshold values are stimulation thresholds expressed as fractions of the stimulation threshold of the action potential. Threshold values for e.p.s.p.s are for e.p.s.p.s with times to peak amplitudes of 7–10 ms post-stimulus.

of their  $E_{i.p.s.p.}$  values on the extracellular potassium concentration, and their insensitivity to bicuculline (Table 1; Howe, Sutor & Zieglängsberger, 1986).

In some neurones, stimulation of superficial cortical layers also evoked e.p.s.p.s with times to peak of 25–80 ms. These late e.p.s.p.s were always evoked at higher stimulation intensities than the commonly evoked e.p.s.p.s with times to peak of 7–10 ms, but disappeared as the stimulation intensity was increased further and i.p.s.p.s were recruited. Thus late e.p.s.p.s were usually only evoked over a narrow range of stimulation intensities. These late e.p.s.p.s exhibited very sharp stimulation thresholds and were often irregular in shape and somewhat variable in amplitude. In a few neurones, however, in which i.p.s.p.s were absent or weak at subthreshold stimulation intensities, late e.p.s.p.s could be evoked over a wider range of intensities and their amplitudes were consistent enough to allow the effect of baclofen on these late e.p.s.p.s to be tested reliably.

#### *The effect of baclofen on e.p.s.p.s*

DL- or L-baclofen decreased the amplitude of e.p.s.p.s in fifty-seven of sixty neurones tested (Fig. 1). Ionophoretic applications of 50–100 nA for 10–30 s resulted in 29–86 % reductions of e.p.s.p amplitudes ( $52 \pm 16$  %,  $n = 10$ ). Addition of baclofen (10 nM to 200  $\mu$ M, see below) to the superfusion medium resulted in 23–100 %

reductions of e.p.s.p. amplitudes ( $65 \pm 25\%$ ,  $n = 44$ ). Applications of baclofen from micropipettes by pressure ejection produced similar reductions of e.p.s.p.s ( $n = 3$ ). Baclofen-induced reductions of e.p.s.p. amplitudes were associated with 10–80% increases in e.p.s.p. stimulation thresholds ( $31 \pm 15\%$ ; for the calculation of percentage reductions of e.p.s.p. amplitudes, the largest stimulation intensities that did not evoke i.p.s.p.s were used; the mean reduction of the amplitude of such e.p.s.p.s was 11 mV). Baclofen's percentage reductions of e.p.s.p. amplitudes were independent of

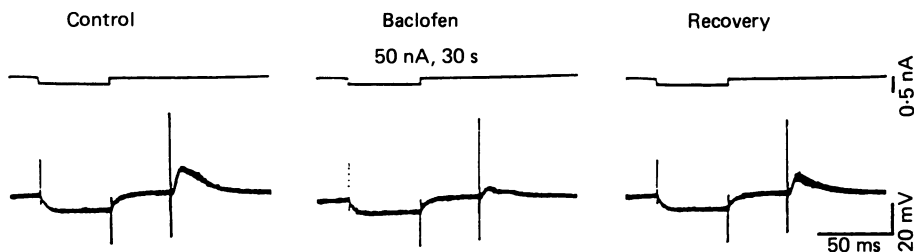


Fig. 1. Ionophoretically applied baclofen depresses e.p.s.p.s. Voltage transients in response to hyperpolarizing current injections (0.2 nA, 50 ms) and e.p.s.p.s evoked with 5 V stimulation (0.25 Hz) before (left), during (middle), and 3.5 min after (right) the ionophoretic ejection of baclofen (50 nA, 30 s). Each picture represents five successive superimposed oscilloscope sweeps. Note the small hyperpolarization (3 mV) and decrease in resistance (20%) during baclofen. Control resting  $E_m = -81$  mV. The recording electrode was filled with potassium acetate; the superfusion medium contained 5.00 mM-potassium. On this and all subsequent Figures, the current monitor was set such that zero current corresponds to the zero potential and recordings were made in bridge mode unless stated otherwise.

$E_m$  over the range of  $E_m$  values tested ( $-60$  to  $-110$  mV). The effects of baclofen on late e.p.s.p.s were quantitatively similar to its effects on e.p.s.p.s with times to peak of 7–10 ms. Full or partial reversibility of baclofen's reductions of e.p.s.p.s was demonstrated for thirty-five and twelve neurones, respectively. For ten neurones, the impalement was lost before recovery was demonstrated.

#### *The effect of baclofen on i.p.s.p.s*

DL- or L-baclofen depressed f-i.p.s.p.s in thirty-seven of thirty-nine neurones tested and l-i.p.s.p.s in seventeen of seventeen neurones tested (Fig. 2). The amplitudes of f-i.p.s.p.s and l-i.p.s.p.s that were evoked with suprathreshold stimulation intensities ( $1.3 \times$  threshold to  $2 \times$  threshold) were reduced 22–100% and 43–100%, respectively (median reductions: 80 and 93%). Determinations of the stimulation threshold of f-i.p.s.p.s were performed in the absence and presence of baclofen for twenty neurones. Baclofen increased the stimulation threshold of f-i.p.s.p.s in each neurone (16–275%, median: 54%). The stimulation threshold for evocation of l-i.p.s.p.s was increased during baclofen in each of six neurones tested (128–200%, median: 146%). Baclofen was applied by addition to the superfusion medium on all but two of the thirty-nine neurones (baclofen concentration: 100 nM to 200  $\mu$ M, see below), and recovery from baclofen's depression of i.p.s.p.s typically took 30–40 min. Full or partial recovery of baclofen-induced depressions of i.p.s.p.s was demonstrated for f-i.p.s.p.s in sixteen and eight neurones and for l-i.p.s.p.s in six and five neurones.

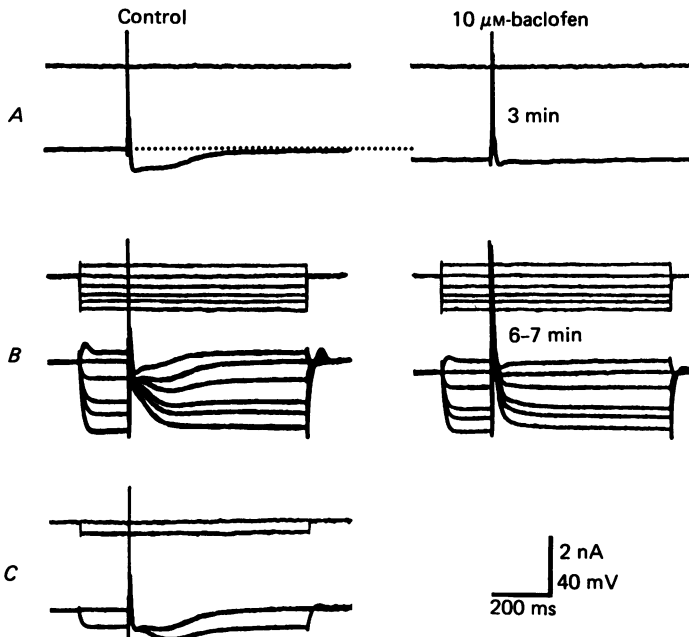


Fig. 2. Baclofen depresses i.p.s.p.s. Left, action potential and biphasic hyperpolarizing potential sequence composed of f- and l-i.p.s.p.s (A,  $2 \times$  threshold stimulation, 0.167 Hz). The l-i.p.s.p.s can be seen most clearly when  $E_m$  is shifted to the reversal potential of f-i.p.s.p.s (C) with hyperpolarizing current injections (0.4 nA, 800 ms). Right, 10  $\mu\text{M}$ -baclofen hyperpolarized  $E_m$  from  $-56$  to  $-62$  mV (A) and blocked both f- and l-i.p.s.p.s at each  $E_m$  value tested (B). Each trace is two superimposed sweeps.  $\text{KMeSO}_4$  recording electrode, 2.50 mm-potassium media.

Baclofen's depressions of i.p.s.p.s were voltage independent between  $-50$  and  $-110$  mV (Figs. 2, 3 and 5 A). Baclofen did not alter  $E_{\text{i.p.s.p.}}$  values of either f-i.p.s.p.s or l-i.p.s.p.s (determinations performed in the absence and presence of baclofen for twenty-nine and seventeen neurones, respectively). Baclofen did, however, markedly and reversibly reduce the conductance increases associated with f-i.p.s.p.s and l-i.p.s.p.s on each neurone tested ( $n = 9$  and 5, respectively). On these same neurones, baclofen increased resting  $g_N$  and decreased direct excitability (Fig. 3). These latter effects recovered minutes before recovery of baclofen's depressions of the conductance increases during i.p.s.p.s (Fig. 4).

#### *Effects of baclofen on synaptic excitability*

DL- or L-baclofen increased the stimulation threshold for orthodromically evoked action potentials in thirty-nine of forty-four neurones tested (6–233 %, median increase: 27 %). Full or partial reversibility of these effects was demonstrated for twenty-six and two neurones, respectively. Thus, the predominant effect of baclofen was to reduce synaptic excitability (Figs. 4 C and 5 B). At suprathreshold stimulation intensities, however, there was often an increase in synaptic excitability, as evidenced by an increase in the number of synaptically evoked action potentials (Fig. 5 A). In cells in which i.p.s.p.s were particularly prominent at subthreshold stimulation

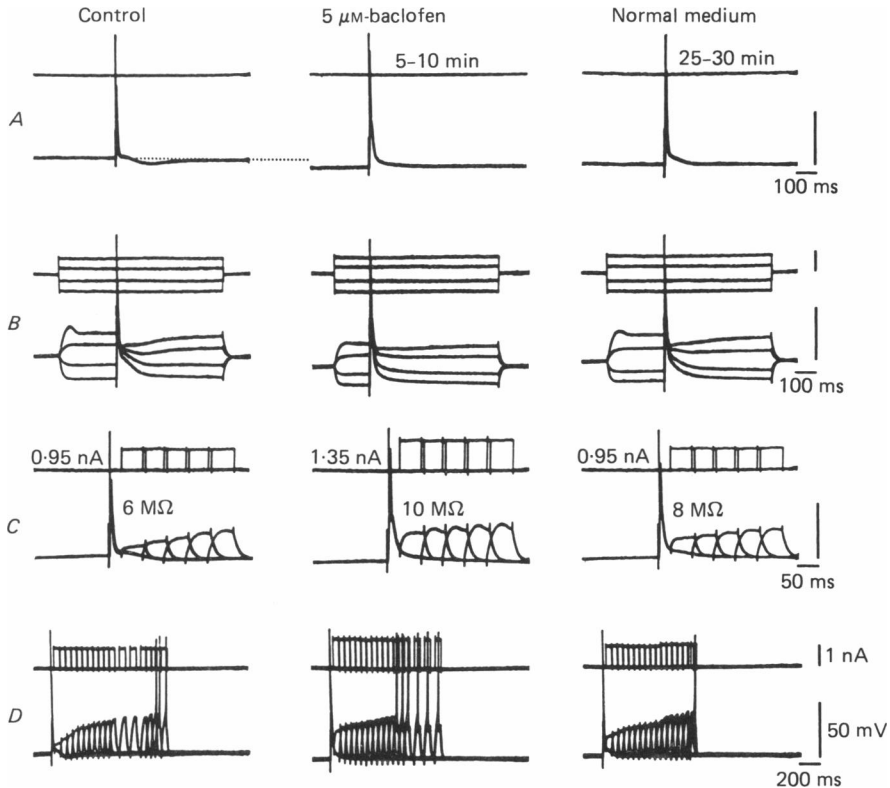


Fig. 3. Baclofen reduces the conductance increases during i.p.s.p.s. Left column: *A*, action potentials and depolarizing f-i.p.s.p.-hyperpolarizing l-i.p.s.p. potential sequences evoked with  $2 \times$  threshold stimulation (0.167 Hz) at resting  $E_m$  ( $-76$  mV, dotted line). *B*, same stimulation applied after shifting  $E_m$  with current injections. *C*, a 60 ms depolarizing current step of a magnitude just necessary to evoke an action potential in the absence of a stimulus was applied at various post-stimulus times to obtain a relative measurement of  $R_N$  during the i.p.s.p. sequences. Values in megaohms are the resistances calculated for the first post-stimulus measurement. *D*, same as *C*, but on a slower time sweep to show the duration of the post-stimulus inhibition and the reduction in the duration of the post-stimulus blockade of the directly evoked action potential during baclofen. The voltage response to the current step in the absence of the stimulus was checked every third post-stimulus time (note the increase in current magnitude during the measurement sequence depicted in the bottom right because the control injection failed to produce an action potential). Middle column: a 10 min application of  $5 \mu\text{M}$ -baclofen hyperpolarized  $E_m$  by 6 mV and increased  $R_N$  by 26%. The magnitude of the current necessary to evoke an action potential was increased from 0.95 to 1.35 nA during baclofen (*C*). Baclofen produced a voltage-independent reduction of f- and l-i.p.s.p.s (*B*) and, in contrast to the decrease in resting  $R_N$  during baclofen,  $R_N$  post-stimulus is increased (*C* and *D*). Right column: partial recovery 25–30 min after returning to normal medium. Note different time calibrations for *A*, *B*, *C* and *D*. Each trace is two oscilloscope sweeps in *A* and *B* and one sweep in *C* and *D*.  $\text{KMeSO}_4$  recording electrode, 2.50 mM-potassium media.



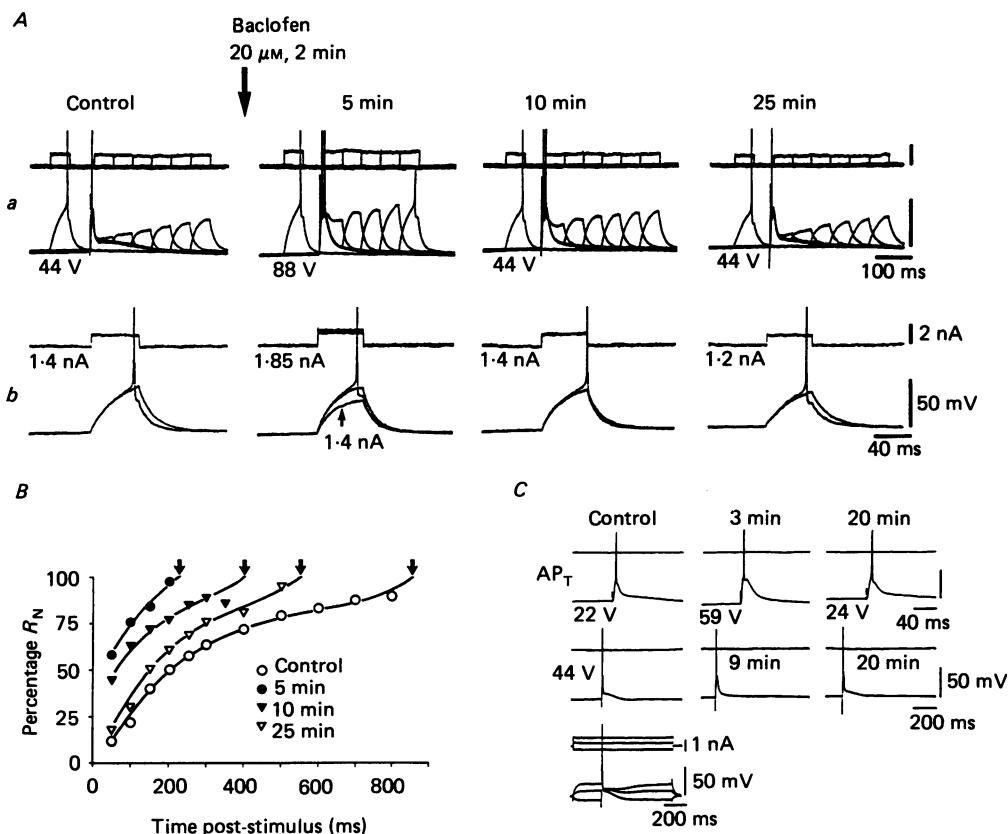


Fig. 4. Baclofen's reductions of i.p.s.p.s outlast its reductions of resting  $R_N$ . *Aa*, determination of relative  $R_N$  as a function of post-stimulus time before and at the indicated times after baclofen with a protocol similar to that depicted in Fig. 3. Stimuli (0.125 Hz) were applied at the intensities noted below each panel (switched current-clamp recording (1.2 kHz), therefore one action potential is cut). *Ab*, responses to current injections in the absence of a stimulus on a faster time sweep. Current values are those that produced the action potential (except as indicated by arrow). Other voltage transients are in response to current injections 0.05 nA less in magnitude. A 2 min application of 20  $\mu$ M-baclofen resulted in a 3 mV hyperpolarization and a significant decrease in resting  $R_N$  (*Ab*, 5 min).  $R_N$  post-stimulus is markedly increased relative to control, however, even with a higher stimulation intensity (88 V; *Aa*, 5 min). This reduction of the conductance increases during the i.p.s.p. sequences persists at a time (*Aa*, 10 min) when resting  $E_m$  and pre-stimulus voltage responses to depolarizing current injections have returned to their control values (*Ab*, 10 min). *B*, plot of the full set of data from the measurements in *A*.  $R_N$  post-stimulus was expressed as a percentage of  $R_N$  pre-stimulus which was estimated from the voltage transients that did not produce an action potential in *Ab*. Arrows indicate the post-stimulus time at which the directly evoked spike was not blocked. *C*, action potentials (bridge mode) evoked at threshold stimulation intensities (top row,  $AP_T$  as indicated) and with 44 V stimulation (bottom) at the indicated times relative to the start of the baclofen application. Bottom left, 44 V stimulation applied after displacing  $E_m$  with current injections. Control resting  $E_m = -87$  mV. KMeSO<sub>4</sub> recording electrode, 2.50 mM-potassium media.

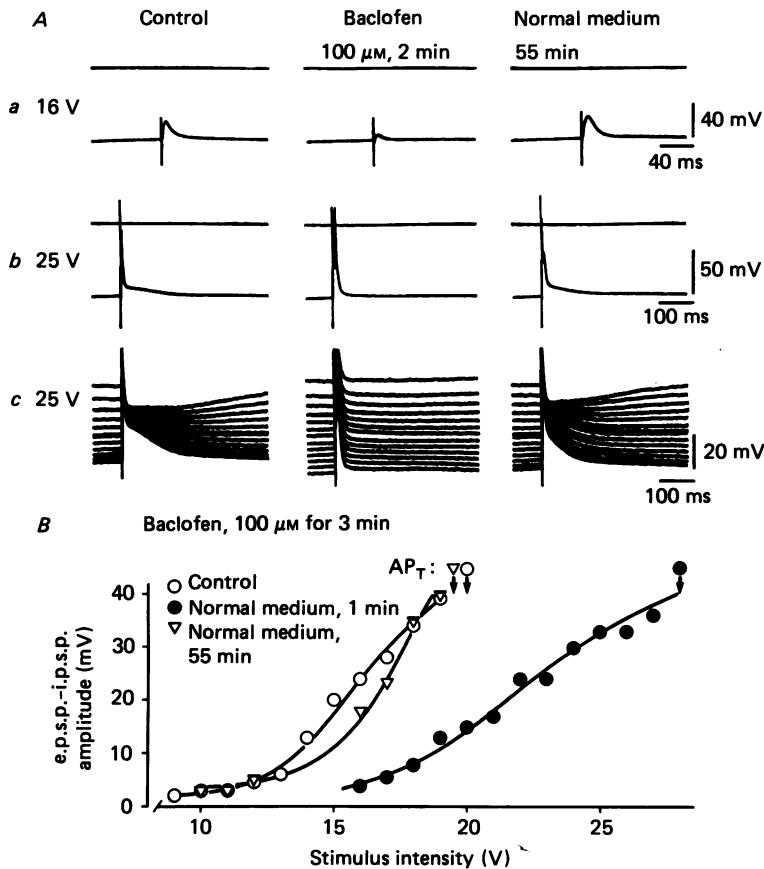


Fig. 5. Effect of baclofen on synaptic excitability. *Aa*, left, depolarizing e.p.s.p.-i.p.s.p. sequence evoked at subthreshold stimulation (16 V, 0.25 Hz; resting  $E_m = -84$  mV). This potential sequence is reduced in amplitude during a 2 min application of 100  $\mu$ M-baclofen (top middle). The stimulation intensity required to produce one action potential increased from 17 to 20 V. Suprathermal stimulation (25 V) evokes prominent depolarizing f-i.p.s.p.s before baclofen (*Ab*, left) which reverse in polarity at  $-70$  mV (*Ac*, left: switched current-clamp mode current traces not shown). The biphasic nature of the hyperpolarizing i.p.s.p. sequence indicates that this intensity (25 V) also evokes l-i.p.s.p.s. Baclofen blocks these i.p.s.p.s at all  $E_m$  values tested (*Ac*, middle) and 25 V stimuli during baclofen produce a burst of three action potentials (*Ab*, middle). Right, recovery 55 min after switching back to normal medium. Each trace is two superimposed sweeps. *B*, plot of stimulation intensity *vs.* amplitude of the depolarizing e.p.s.p.-i.p.s.p. sequence to action potential threshold ( $AP_T$ , arrows) from measurements made on another neurone (resting  $E_m = -86$  mV). A 3 min application of 100  $\mu$ M-baclofen caused a marked and reversible shift to the right of the input-output curve 1 min after baclofen. Potassium acetate recording electrodes, 5.00 mM-potassium media.

intensities, the increases of action potential threshold produced by baclofen were modest, or this threshold was even reversibly decreased (6–20 %,  $n = 4$ ).

#### Effects of baclofen on $E_m$ and $R_N$

Applications of DL- or L-baclofen produced hyperpolarizations ( $1$ – $12$  mV,  $4 \pm 2$  mV) in forty of seventy-one neurones. Complete reversibility of these hyperpolarizations

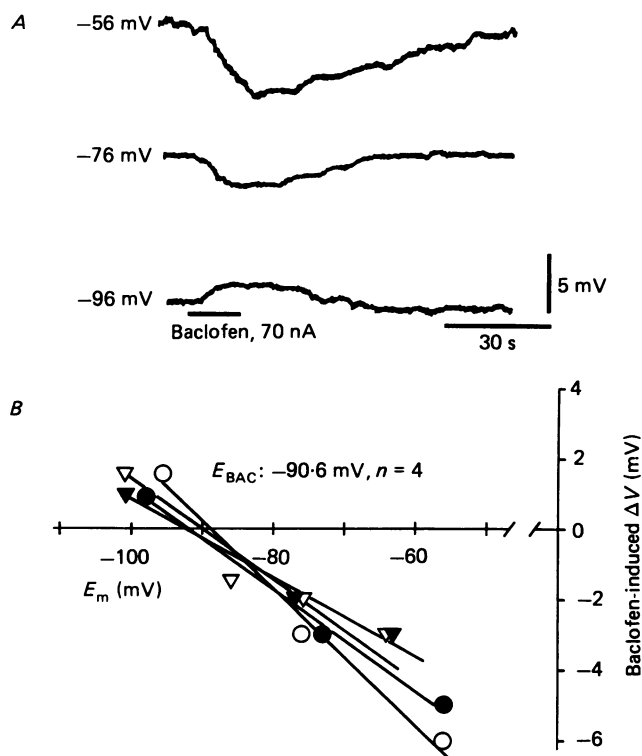


Fig. 6. Reversal potential of baclofen-induced changes in  $E_m$ . *A*, changes of  $E_m$  produced by the ionophoretic application of baclofen at resting  $E_m$  ( $-76 \text{ mV}$ ) and after displacing  $E_m$  to the indicated values with constant-current injection. *B*, plots for determination of  $E_{BAC}$  for the cell in *A* and for three other cells. The range of  $E_{BAC}$  values is  $-89$  to  $-92 \text{ mV}$ . All determinations were made in  $5.00 \text{ mM}$ -potassium media and with potassium acetate recording electrodes.

was demonstrated for thirty-five neurones. Depolarizations in response to baclofen were never observed when baclofen was applied at resting  $E_m$ . In each of seven neurones on which ionophoretic applications of both GABA and baclofen were made at resting  $E_m$ , GABA produced depolarizations and baclofen produced hyperpolarizations.

The amplitude of baclofen-induced hyperpolarizations was increased when  $E_m$  was depolarized, and in four neurones it was possible to hyperpolarize  $E_m$  far enough to reverse these hyperpolarizations. Determinations of the reversal potential ( $E_{BAC}$ ) of  $E_m$  changes produced by ionophoretically applied baclofen were between  $-89$  and  $-92 \text{ mV}$  in  $5 \text{ mM}$ -potassium medium (Fig. 6). Estimations of  $E_{BAC}$  were also made from the intersection point of current-voltage relation plots determined in normal and in baclofen-containing media for another fifteen neurones (Ginsborg, 1967). Mean  $E_{BAC}$  values so estimated were  $-85 \pm 6 \text{ mV}$  and  $-97 \pm 5 \text{ mV}$  in  $5.00$  and  $2.50 \text{ mM}$ -potassium media, respectively ( $n = 8$  and  $7$ ; values significantly different from each other,  $P < 0.005$ ). The amplitude of baclofen-induced hyperpolarizations was increased when the potassium concentration of the medium was reduced from  $5.00$  to  $1.25 \text{ mM}$  and the  $E_{BAC}$  values estimated from the intersection point of current-voltage relations plots were shifted by  $-21$  and  $-24 \text{ mV}$ .

For twenty-nine of the forty neurones hyperpolarized by baclofen, reductions in  $R_N$  were measured during these hyperpolarizations (10–58 %,  $26 \pm 10$  %). These  $R_N$  decreases were not solely the consequence of the anomalous rectification inherent to the neurones, because significant decreases in  $R_N$  remained when constant current was injected to shift  $E_m$  back to its pre-application value. Furthermore, in only a few cases were the hyperpolarizations large enough for the inward rectifying behaviour of the cells to influence significantly the magnitude of the  $R_N$  decreases measured. The most exceptional case was the 58 % reduction in  $R_N$  measured in a neurone that baclofen hyperpolarized from  $-69$  to  $-81$  mV. In seven neurones,  $R_N$  decreases were not detected during the small hyperpolarizations produced by baclofen, and reversible decreases in  $R_N$  (15–30 %) were measured in six neurones that were not hyperpolarized by baclofen.

Current-voltage relation determinations in normal and in baclofen-containing media were made for fifteen neurones. In four cases, comparisons of the resultant 10–30 point plots suggested that the absolute decrease in  $R_N$  produced by baclofen was greatest at hyperpolarized  $E_m$  values. In the other eleven cases however, these comparisons gave no indication that baclofen-induced  $R_N$  changes were voltage dependent. Determinations of the dependence of  $R_N$  on  $E_m$  were made (as described in the first section of the Results) in both normal and in baclofen-containing media for eight neurones. These measurements also did not indicate that there was any significant or consistent voltage dependence of the  $R_N$  decreases measured during baclofen.

Importantly, in virtually every neurone tested, the hyperpolarizations and decreases in  $R_N$  produced by baclofen recovered before the baclofen-induced depressions of post-synaptic potentials (Fig. 8). The effects of baclofen on synaptically evoked potentials often outlasted its effects on  $E_m$  and  $R_N$  by several minutes.

#### *Effects of baclofen on neurones recorded in voltage clamp*

Outward currents in response to ionophoretically applied baclofen or to short bath applications of high concentrations of baclofen were recorded in somatic voltage clamp at holding potentials of  $-55$  to  $-65$  mV in three of four neurones tested (Fig. 7). These outward currents were associated with 10–20 % increases in  $g_N$ . The currents had kinetics similar to the baclofen-induced hyperpolarizations recorded in current clamp from the same neurones and were of sufficient magnitude to account for the magnitude of these hyperpolarizations. Applications of baclofen did not produce measurable currents in either of two cells clamped at a holding potential of  $-80$  mV in 5 mM-potassium medium (Fig. 10).

#### *Effects of baclofen on direct excitability*

DL- or L-baclofen increased the magnitude of the depolarizing current necessary to evoke an action potential for twenty-three of twenty-seven neurones tested (11–50 %,  $30 \pm 13$  %). Full reversibility of these reductions in direct excitability was demonstrated for seventeen neurones. Baclofen did not change the threshold voltage for action potential generation and had no noticeable effects on action potential rise time or duration. Increases of direct excitability during baclofen were never observed.

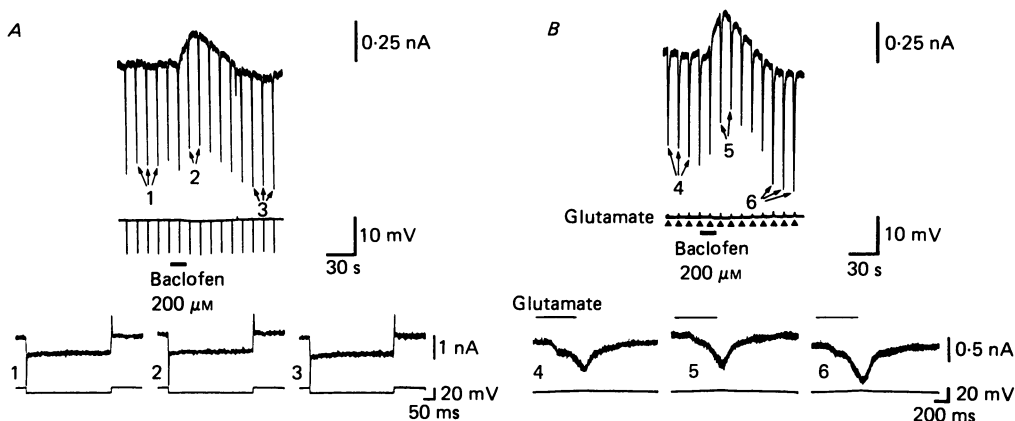


Fig. 7. Outward currents underlie the hyperpolarizations produced by baclofen. *A*, a 15 s application of  $200\ \mu\text{M}$ -baclofen (bars below voltage trace) produces an outward current (upper trace) in a neurone voltage clamped at a holding potential of  $-60\ \text{mV}$  (switching frequency:  $1.7\ \text{kHz}$ ). Downward deflexions on the current trace are inward current responses to  $10\ \text{mV}$  hyperpolarizing command steps. The apparent magnitudes of these currents are cut by the frequency response of the pen writer; however, comparison of the amplitudes of the indicated responses that were photographed from the oscilloscope (below) demonstrates that during the baclofen-induced outward current there is a 10–15 % increase in  $g_{\text{N}}$ . *B*, another application of baclofen ( $200\ \mu\text{M}$ , 15 s, bar) produces a similar outward current (holding potential:  $-55\ \text{mV}$ ). Downward deflexions on the current trace represent inward currents evoked in response to ionophoretic applications of L-glutamate ( $90\ \text{nA}$ ,  $700\ \text{ms}$ , at arrowheads below voltage trace). These inward currents were unchanged during the baclofen-induced outward current. The indicated inward currents are superimposed in the photographs below (L-glutamate applications indicated by bars above current traces). The outward currents evoked by baclofen in this neurone had kinetics that were similar to those of the hyperpolarizations produced by similar baclofen applications made during current-clamp recording at resting  $E_{\text{m}}$  ( $-66\ \text{mV}$ ; see Fig. 8*D*).  $\text{KMeSO}_4$  recording electrode,  $2.50\ \text{mM}$ -potassium media.

#### *Effects of baclofen on responses evoked by excitatory amino acids or GABA*

Applications of baclofen that markedly reduced e.p.s.p.s produced no or only slight ( $< 20\%$ ) reductions of depolarizations evoked by the ionophoretic application of L-glutamate (eight neurones tested, Figs. 8 and 9). The percentage reductions of L-glutamate-induced depolarizations that were observed were similar in magnitude to the percentage reductions of  $R_{\text{N}}$  measured during the baclofen applications; and, like  $R_{\text{N}}$ , depolarizations produced by L-glutamate recovered to control values minutes before the recovery of baclofen-induced depressions of e.p.s.p.s. Applications of baclofen that markedly reduced the amplitudes of e.p.s.p.s also did not significantly reduce depolarizations produced by L-aspartate or NMDA (four applications on three neurones, Fig. 9). Applications of baclofen that markedly reduced e.p.s.p.s recorded in current clamp, produced no change in the amplitude of L-glutamate-induced inward currents recorded from these same neurones in somatic voltage clamp ( $n = 2$ , Fig. 7*B*).

Baclofen applications that completely and reversibly blocked f.i.p.s.p.s evoked with suprathreshold stimulation intensities had no effect on depolarizations produced in the same neurones by ionophoretic applications of GABA ( $n = 3$ ). The decreases

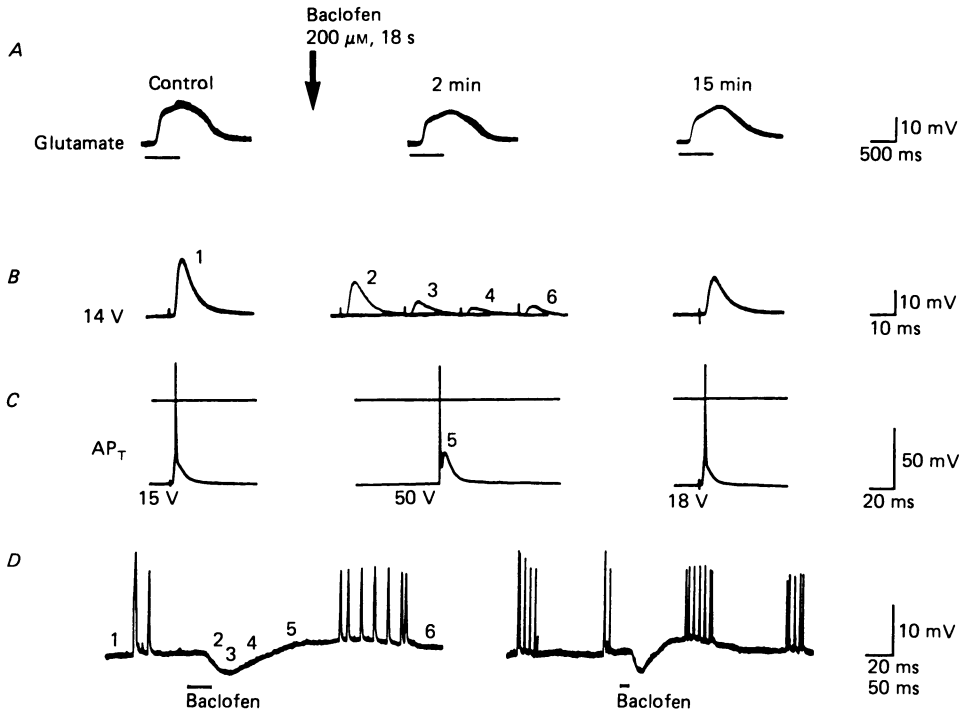


Fig. 8. Baclofen's depressions of e.p.s.p.s can be dissociated from its effects on  $E_m$  and on depolarizations evoked by L-glutamate. *A* (glutamate), depolarizing responses (each four superimposed sweeps) to ionophoretic applications of L-glutamate (80 nA for 700 ms, bars) before and at the indicated times after an 18 s application of 200  $\mu$ M-baclofen (application time indicated by the bar below the chart records shown at the bottom of the Figure). *B* (14 V), e.p.s.p.s evoked with 14 V stimulation (0.10 Hz) at the times indicated on the chart record of  $E_m$  on *D*, left (record *D*, right, depicts the same application at a different time calibration; pen-writer deflexions in response to stimulation-evoked potentials have been touched out of the records to enhance the clarity of presentation). Picture 1 and the e.p.s.p.s 15 min after baclofen are four superimposed sweeps. Pictures 2, 3, 4 and 6 are each one sweep. In order that the amplitudes of the e.p.s.p.s can be better compared, the potentials were replayed at the same offset level when the photograph was made. *C*, action potentials at the indicated threshold stimulation intensities ( $AP_T$ ). Note that  $AP_T$  is still increased more than 3-fold after  $E_m$  has repolarized (picture 5) and that e.p.s.p.s remain markedly reduced at a time (6) when the depolarizations evoked by L-glutamate are hardly affected. This Figure depicts an earlier baclofen application made on the neurone that is depicted in Fig. 7.

in  $R_N$  associated with these depolarizations were also unaffected by baclofen. Importantly, baclofen (three applications on two neurones) had no effect on inward currents produced by GABA during voltage-clamp recordings (Fig. 10).

#### *Reproducibility and concentration dependence of baclofen effects*

The reproducibility of baclofen's effects on post-synaptic potentials and on  $E_m$  and  $R_N$  were most easily demonstrated when baclofen was applied by ionophoresis, as the effects were then relatively short-lived. The prolonged depressions of post-synaptic potentials produced by bath applications of baclofen limited the possibilities for

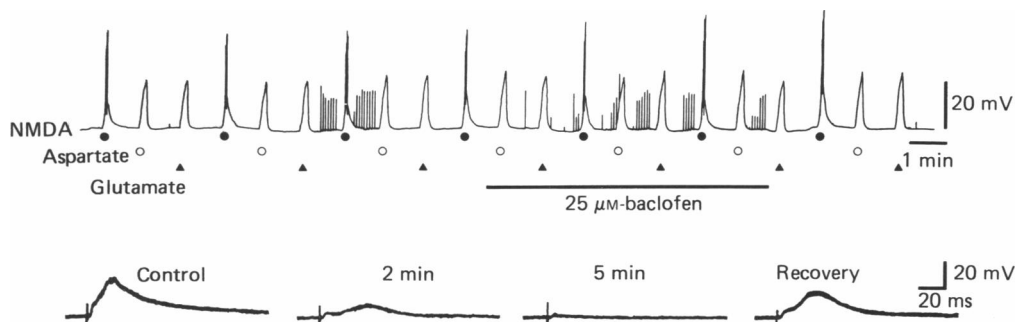


Fig. 9. Baclofen does not depress responses to ionophoretically applied excitatory amino acids. Chart records of depolarizing responses to ionophoretic applications of NMDA (75 nA for 6 s), L-aspartate (100 nA for 10 s), or L-glutamate (100 nA for 10 s). Action potentials during the NMDA depolarizations are cut by the frequency response of the pen writer. Upward vertical lines are pen-writer deflexions in response to post-synaptic potentials evoked with various stimulation intensities. Pictures below the chart record depict e.p.s.p.s (note presence of late e.p.s.p.s which are seen most clearly in the picture labelled 2 min) that were evoked with 11 V stimulation before, 2 and 5 min into, and 20 min after the indicated 7 min application of 25  $\mu$ M-baclofen (six, two, two and five successive superimposed sweeps, respectively). In contrast to the almost complete depressions of e.p.s.p.s, depolarizations produced by either of the three excitatory amino acids are unchanged by baclofen. Resting  $E_m = -84$  mV. Potassium acetate recording electrode, 5.00 mM-potassium media.

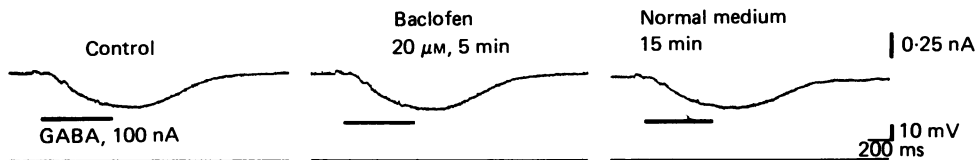


Fig. 10. Baclofen does not reduce inward currents evoked by ionophoretically applied GABA. Inward currents recorded in somatic voltage clamp (switching frequency: 10.8 kHz) at a holding potential of  $-80$  mV in response to ionophoretic applications of GABA (bars, 100 nA for 600 ms). Each trace is the digitized computer average of five successive responses recorded before, 5 min into and 15 min after a 6 min application of 20  $\mu$ M-baclofen. The resting  $E_m$  of the neurone was  $-78$  mV and when the same GABA applications were made in current clamp they produced 7–8 mV depolarizations. This same baclofen application completely and reversibly blocked suprathreshold f.i.p.s.p.s recorded from the neurone in current clamp. Potassium acetate electrode, 5.00 mM-potassium media.

repeated testing; however, more than one such application was made on eighteen neurones and the results from these neurones also demonstrated convincingly the reproducibility of baclofen's effects and provided no evidence for significant desensitization.

In order to increase the possibility of demonstrating full recovery, high concentrations of baclofen were applied for short times in some experiments. In our experience, however, steady-state responses to changes in the ionic composition of the medium or to bath-applied baclofen are only reached after application times of 5–10 min with our superfusion system. Therefore only applications of baclofen-

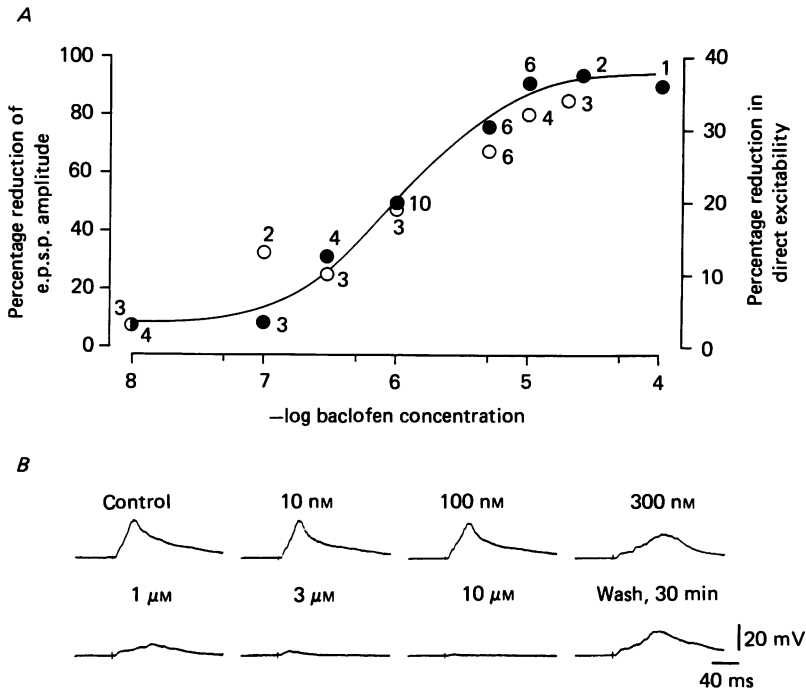


Fig. 11. The  $\text{EC}_{50}$  for baclofen's reductions of e.p.s.p.s and direct excitability is approximately  $1 \mu\text{M}$ . *A*, concentration-response curves:  $-\log$  baclofen concentration *vs.* percentage reduction of e.p.s.p. amplitudes (●, left) or percentage reduction of direct excitability (○, right; calculated from the percentage increase in the depolarizing current magnitude necessary to produce an action potential). Points represent the mean percentage reductions of each parameter calculated from the data obtained during baclofen superfusions  $\geq 5$  min in duration. The number of such applications of each concentration is indicated by the number next to each data point (the data from one application of  $3 \mu\text{M}$ -baclofen were averaged together with the data from five applications of  $5 \mu\text{M}$ -baclofen to yield the points plotted at  $5 \mu\text{M}$ ). The curve was fitted by eye through the e.p.s.p. data. The approximate  $\text{EC}_{50}$  for each action of baclofen is  $1 \mu\text{M}$ . Note the difference between the maxima of the scales. *B*, e.p.s.p.s evoked with 12.5 V stimulation before (control) and during applications of the indicated concentrations of baclofen, and 30 min after returning to normal medium (wash). Note the two-component nature of the e.p.s.p.s. Late e.p.s.p.s could be evoked in control at all stimulation intensities between 11 V and action potential threshold (14 V) and were of consistent amplitude at each intensity. The superfusions with baclofen-containing media were 19–28 min in duration and were made in consecutive ascending order of concentration without intermediate washes. The depicted e.p.s.p.s were all evoked after at least 12 min of superfusion with the indicated baclofen concentration. Membrane potential values were (from upper left to lower right):  $-83$ ,  $-83$ ,  $-85$ ,  $-87$ ,  $-89$ ,  $-91$ ,  $-92$  and  $-87$  mV. Potassium acetate recording electrode, 2.5 mM-potassium media.

containing medium of  $\geq 5$  min were considered in the analysis of the concentration dependence of baclofen's actions.

Fig. 11*A* presents a concentration-response curve for baclofen's depression of e.p.s.p.s based on the mean data from all applications of  $\geq 5$  min. The results indicate that the threshold concentration for baclofen's reductions of e.p.s.p. amplitudes is between 10 and 100 nM and that the  $\text{EC}_{50}$  for baclofen's depression of e.p.s.p.s is approximately  $1 \mu\text{M}$ . The results from experiments in which it was possible to make



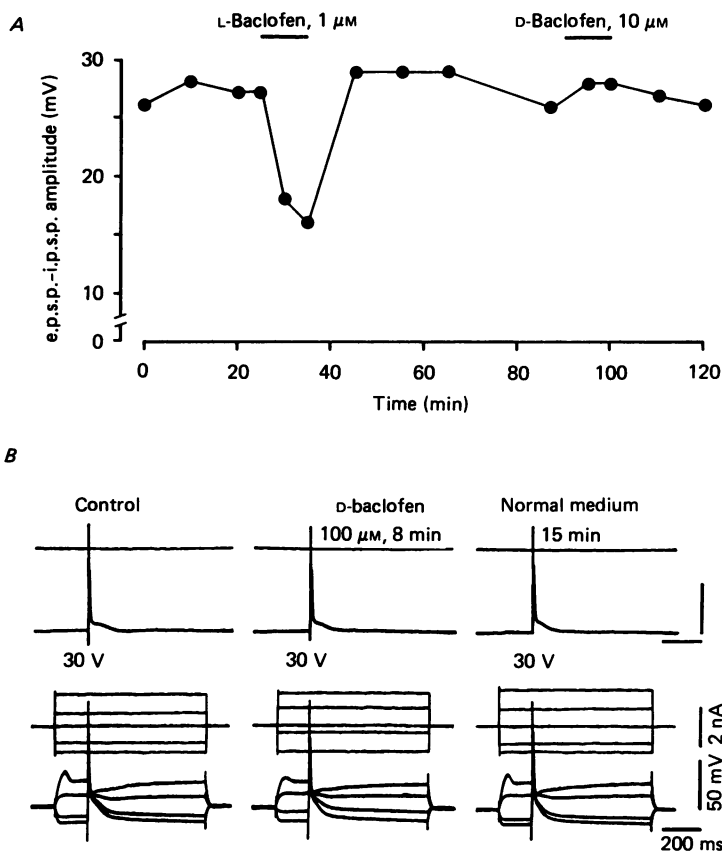


Fig. 12. Baclofen's depressions of e.p.s.p.s and i.p.s.p.s are stereoselective. *A*, plot of the amplitude of the depolarizing e.p.s.p.-i.p.s.p. sequence evoked at 24 V stimulation (0.8  $\times$  threshold, 0.25 Hz) *vs.* time. Applications of L- or D-baclofen are indicated by the bars above the plot. 1  $\mu$ M-L-baclofen depressed the e.p.s.p.-i.p.s.p. amplitude by 50% and increased the action potential stimulation threshold ( $AP_T$ ) by 20%. D-Baclofen (10  $\mu$ M) did not alter the e.p.s.p.-i.p.s.p. amplitude nor  $AP_T$ . L-Baclofen produced a 3 mV hyperpolarization. Control resting  $E_m$  was  $-84$  mV. *B*, top row, action potentials and depolarizing f.i.p.s.p.-hyperpolarizing l-i.p.s.p. sequences evoked by 30 V stimulation (0.25 Hz) before, during and after a 10 min application of 100  $\mu$ M-D-baclofen. Bottom row, stimuli applied after displacing  $E_m$  with 800 ms current injections. All traces are two superimposed sweeps. 100  $\mu$ M-D-baclofen had no effect on the i.p.s.p. sequence. This D-baclofen application also had no effect on: the complete e.p.s.p.-i.p.s.p. input-output relation;  $AP_T$ ;  $E_m$ ; or direct excitability. Control resting  $E_m = -82$  mV. KMeSO<sub>4</sub> recording electrodes, 2.50 mM-potassium media.

applications of several concentrations on the same neurone support these conclusions drawn from the pooled data (Fig. 11 *B*).

Similar results were obtained with regard to baclofen's depression of i.p.s.p.s. 10–20% increases in i.p.s.p. stimulation thresholds were observed with baclofen concentrations of 100–300 nM. Mean depressions of the amplitudes of suprathreshold f-i.p.s.p.s were 38, 76, 89 and 93% at concentrations of 1  $\mu$ M ( $n = 2$ ), 3–5  $\mu$ M ( $n = 6$ ), 10  $\mu$ M ( $n = 8$ ), and 20–25  $\mu$ M ( $n = 3$ ), respectively. Concentrations of baclofen of 1–5  $\mu$ M reduced the amplitudes of suprathreshold l-i.p.s.p.s by 46–100% ( $n = 5$ ), and

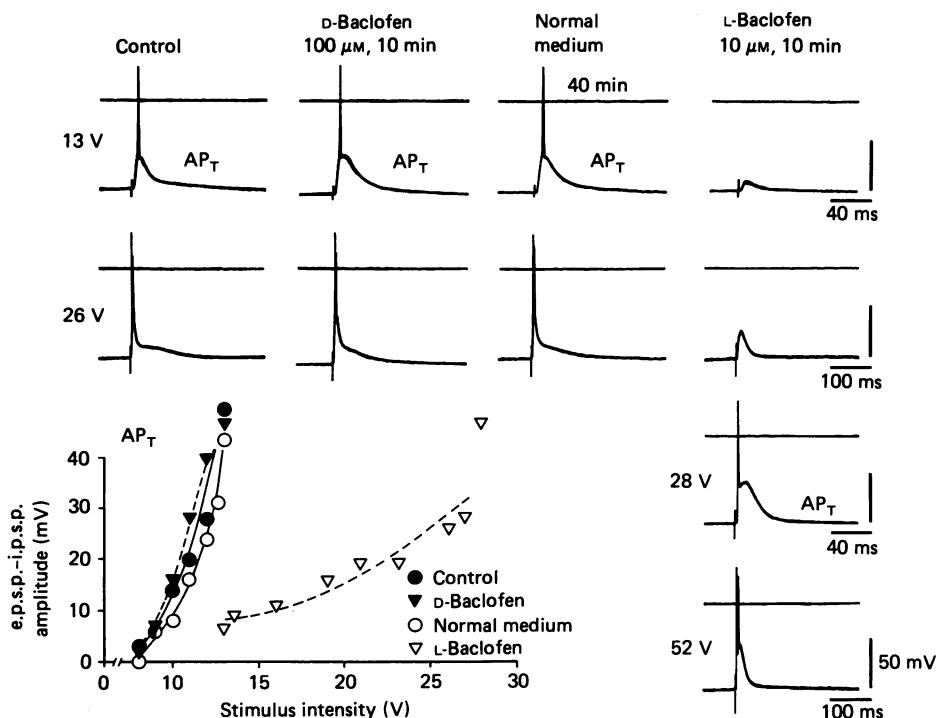


Fig. 13. Baclofen stereoselectively reduces synaptic excitability. Action potentials and post-synaptic potential sequences evoked at the indicated stimulation intensities (in volts; frequency, 0.25 Hz). A 10 min application of 100  $\mu$ M-D-baclofen does not alter the action potential threshold (AP<sub>T</sub>). Depolarizing f-i.p.s.p.s evoked with 26 V stimulation are modestly reduced in amplitude. 10  $\mu$ M-L-baclofen (10 min) more than doubles AP<sub>T</sub> and during L-baclofen there is no evidence of depolarizing f-i.p.s.p.s even with stimulation at 52 V intensity. Each trace is two superimposed sweeps. Inset, plots of the input-output relations for the e.p.s.p.-i.p.s.p. sequence to AP<sub>T</sub> that were determined during superfusion with the indicated media. Resting  $E_m = -83$  mV. KMeSO<sub>4</sub> recording electrode, 2.50 mM-potassium media.

100% depressions of suprathreshold l-i.p.s.p.s were observed for each neurone on which baclofen concentrations of 10–20  $\mu$ M were applied ( $n = 4$ ).

Measurable hyperpolarizations and  $R_N$  decreases were obtained with baclofen concentrations of 100–300 nM. There were no significant differences between the mean amplitudes of baclofen-induced hyperpolarizations and  $R_N$  decreases that were obtained with baclofen concentrations between 1 and 25  $\mu$ M; however, effects of baclofen on  $E_m$  and  $R_N$  were observed more consistently as the baclofen concentration was increased over this range.

In our hands, the increase in the magnitude of the depolarizing current required to produce an action potential was the most sensitive and reliable indicator of baclofen's effects on  $E_m$  and  $R_N$ . Fig. 11A also presents a concentration-response curve for baclofen's depressions of direct excitability based on the mean data obtained from twenty-three applications on twelve neurones. The results indicate that the EC<sub>50</sub> for baclofen's reductions of direct excitability is approximately 1  $\mu$ M.

*Stereoselectivity of baclofen's effects*

The effects of L- and D-baclofen were compared on six neurones. Each of the actions of baclofen was clearly stereoselective. Applications of 1–10  $\mu\text{M}$ -L-baclofen produced depressions of e.p.s.p.s and i.p.s.p.s,  $E_m$  and  $R_N$  changes and decreases in synaptic and direct excitability that were similar in magnitude to those produced on other neurones by the same concentrations of DL-baclofen, whereas applications of D-

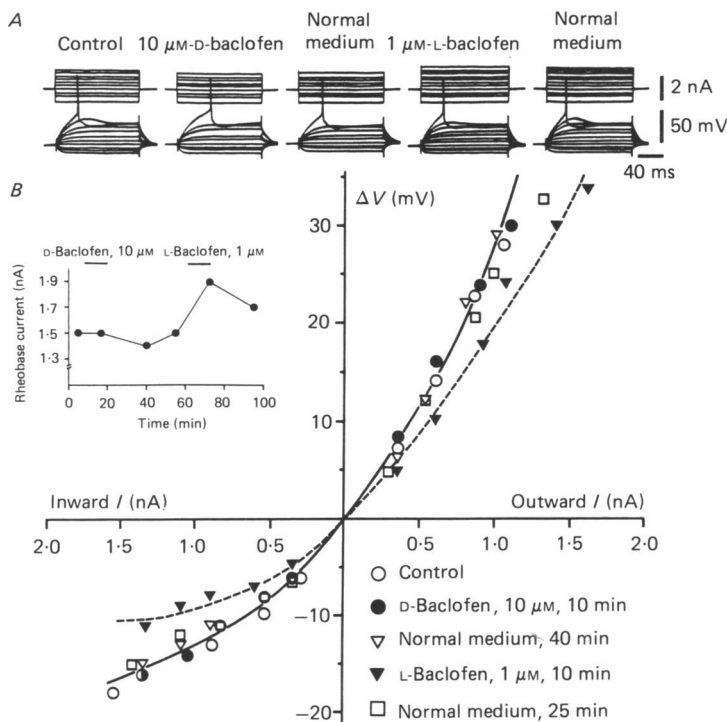


Fig. 14. Baclofen's effects on  $E_m$  and  $R_N$  are stereoselective. *A*, measurements of current-voltage relations (two sweeps each trace except for those with action potential). *B*, current-voltage relations before, during and 40 min after a 12 min application of 10  $\mu\text{M}$ -D-baclofen and during and 25 min after a 12 min application of 1  $\mu\text{M}$ -L-baclofen. Continuous curve was fitted by eye through control and 40 min normal medium data points. Dashed curve was fitted by eye through L-baclofen data points.  $E_m$  (mV) and  $R_N$  ( $\text{M}\Omega$ ) values (determined from the slope at the origin of the curves fitted for each of the five sets of data) were: control, -91 and 20; D-baclofen, -91 and 20; normal medium 40 min, -91 and 19; L-baclofen, -95 and 15; normal medium 25 min, -93 and 18. Inset, plot of rheobase current *vs.* time from determinations made on the same cell. D- and L-baclofen applications are indicated by the bars above the plot.  $\text{KMeSO}_4$  recording electrode, 2.50 mM-potassium media.

baclofen at 10–30-fold higher concentrations produced no significant effects on any of these parameters (Figs. 12, 13 and 14). On each of the five neurones tested, 10 min applications of 100  $\mu\text{M}$ -D-baclofen did not produce any significant effects on  $E_m$ ,  $R_N$ , or direct excitability, and synaptically evoked potentials were either unaltered or were only slightly reduced ( $< 20\%$ ). Because the  $\text{EC}_{50}$  for each action of DL-baclofen is approximately 1  $\mu\text{M}$ , these results indicate that L-baclofen is more than 100-fold

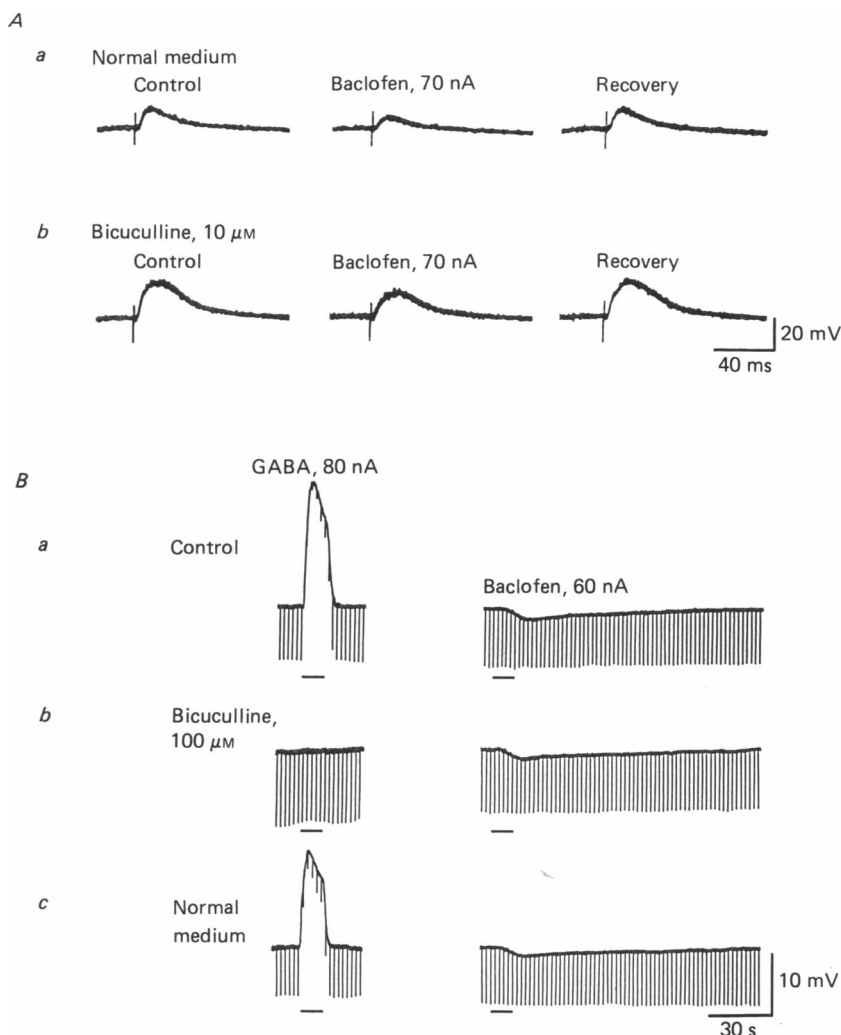


Fig. 15. Baclofen's effects on e.p.s.p.s and on  $E_m$  and  $R_N$  are resistant to blockade by bicuculline. *Aa*, e.p.s.p.s evoked at 5 V stimulation (0.25 Hz) before, during and 90 s after the ionophoretic application of baclofen (70 nA, 15 s) while superfusing with normal medium. Baclofen reduces the amplitude of the e.p.s.p.s by 40%. *Ab*, a 30 min application of 10  $\mu$ M-bicuculline increased the amplitude of e.p.s.p.s evoked with 5 V stimulation, but the relative reduction (36%) of these e.p.s.p.s by baclofen is not significantly changed. Resting  $E_m = -86$  mV. Each trace is two superimposed sweeps. *B*, chart records of  $E_m$  (resting  $E_m = -84$  mV). Downward vertical deflexions are pen-writer responses to voltage transients produced by rectangular hyperpolarizing current injections (0.5 nA, 150 ms). GABA or baclofen were applied by ionophoresis at the indicated ejection currents for 10 s (bars below records). Baclofen applications were made approximately 1 min after the corresponding GABA application while: superfusing with normal medium (control, *Ba*), 20 min after switching to a medium that contained 100  $\mu$ M-bicuculline (*Bb*) and 40 min after returning to normal medium (*Bc*). GABA (control) depolarizes  $E_m$  and produces a marked decrease in  $R_N$ . Both the depolarization and the  $R_N$  decreases are almost completely and reversibly blocked by bicuculline. At the same  $E_m$ , baclofen produces a hyperpolarization and a small decrease in  $R_N$  that are not altered during bicuculline. Potassium acetate recording electrodes, 2.50 mM-potassium media.

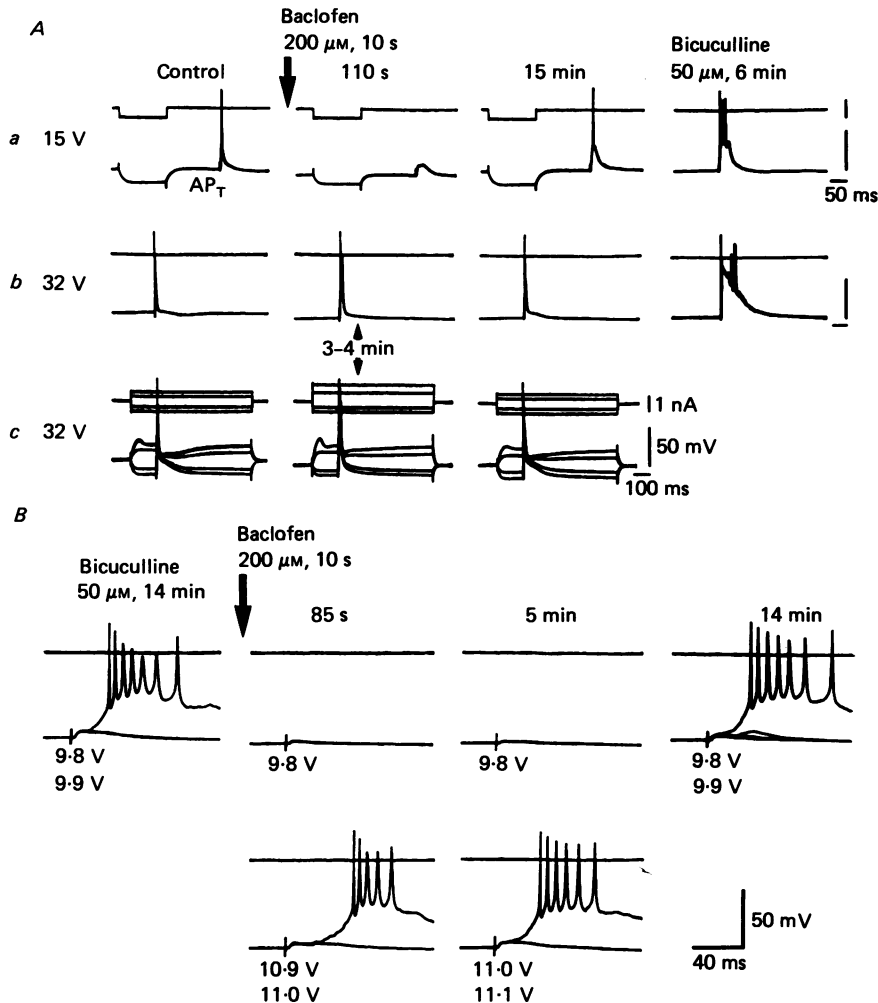


Fig. 16. Baclofen's depressions of synaptic excitability are resistant to blockade by bicuculline. *A*, column 1 (control): *a*, voltage transients in response to hyperpolarizing current injections (0.5 nA, 150 ms) and action potentials at stimulation threshold ( $AP_T = 15$  V); *b*, action potentials and f.i.p.s.p.-l.i.p.s.p. sequences evoked with 32 V stimulation; *c*, stimuli (32 V) applied after displacing  $E_m$  with current injections (800 ms). Column 2, a 10 s application of 200  $\mu$ M-baclofen blocks the stimulation-evoked action potentials ( $AP_T = 19$  V at 130 s) and causes a 5 mV hyperpolarization and a 31 % decrease in  $R_N$  (*a*, 110 s). 3–4 min later, i.p.s.p.s are still markedly depressed (*b* and *c*). Column 3, reversal of these effects of baclofen 15 min post-application. Column 4, the superfusion medium was switched to one that contained 50  $\mu$ M-bicuculline. 6 min later, 15 V stimulation produces five spike bursts (*a*) and 32 V stimulation produces 'giant' e.p.s.p.s during which action potentials are inactivated (*b*). All traces in *A* are two superimposed sweeps. Control resting  $E_m = -76$  mV. *B*, top left, same neurone 14 min into the bicuculline application ( $E_m = -74$  mV). Stimulation at 9.8 V intensity produces 6 mV e.p.s.p.s and a 9.9 V stimulus produces a seven-spike burst of action potentials. Columns 2 and 3, an application of baclofen identical to that in *A* reduces the amplitude of the e.p.s.p.s evoked with 9.8 V stimulation, causes a 6 mV hyperpolarization, and increases the threshold for the action potential burst (bottom row). All these effects are reversible 14 min post-application of baclofen (top right). The responses to 9.8 V stimulation are all four superimposed sweeps. Responses at the other stimulation intensities are each one sweep. KMeSO<sub>4</sub> recording electrode, 2.50 mM-potassium media.

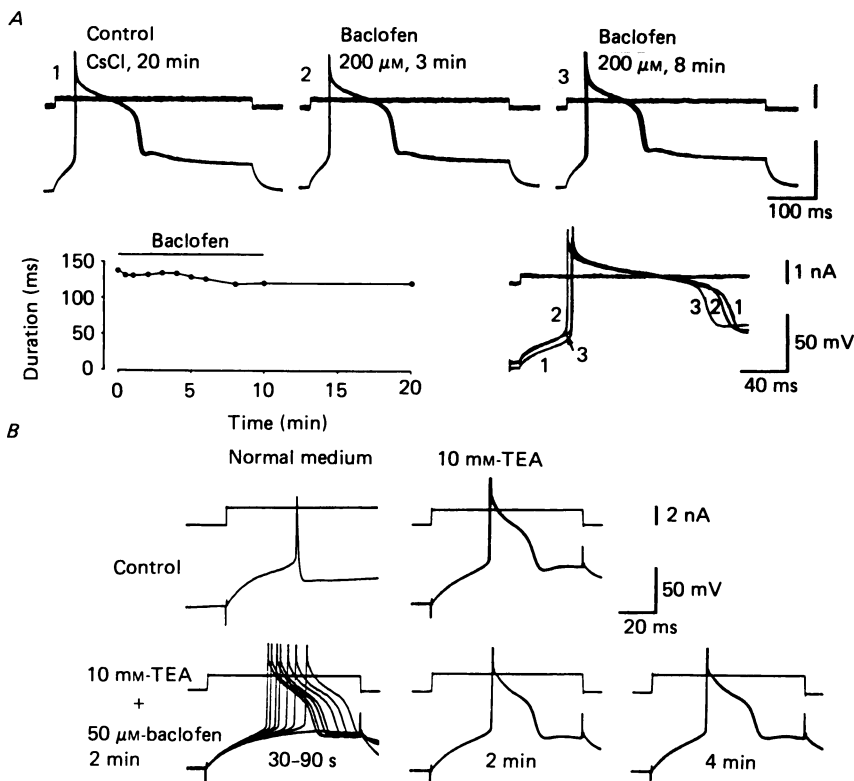


Fig. 17. Baclofen does not reduce the duration of the calcium component of action potentials prolonged with caesium or TEA. *A*, recording with a CsCl electrode. Top left, 20 min into the recording after injecting the cell several times with caesium ions, a depolarizing current step (0.4 nA, 400 ms) evokes action potentials of prolonged duration (135 ms at  $E_m = -30$  mV). Middle and right, action potentials evoked 3 and 8 min into the application of 200  $\mu$ M-baclofen. Each picture in the top row is ten successive superimposed sweeps. Bottom left, plot of action potential duration (at  $E_m = -30$  mV) *vs.* time. The 10 min baclofen application is indicated by the bar. Right, two successive sweeps each at times 1, 2 and 3 in the top row that are superimposed for better comparison (resting  $E_m = -71$ ,  $-68$  and  $-67$  mV, respectively). *B*, top (control), action potentials evoked by depolarizing current injections (1.55 nA) before (left, one sweep) and 13 min after (right, five sweeps) switching to a superfusion medium that contained 10 mM-TEA. Bottom left, superimposed sweeps 30, 50, 62, 70, 78, 82, 86 and 90 s after switching to TEA + 50  $\mu$ M-baclofen medium, action potentials at 30–86 s are left to right and at 90 s the current injection failed to produce an action potential. Middle and right, the current magnitude was increased (to 1.80 nA) to evoke an action potential. The duration of these action potentials was not reduced either 2–4 min after starting the 2 min baclofen application (pictures are four sweeps each). Resting  $E_m = -88$  mV (normal medium),  $-85$  mV (TEA). KMeSO<sub>4</sub> recording electrode, 2.50 mM-potassium media.

more potent than D-baclofen at producing each of the effects observed. The potency of DL- and L-baclofen were not compared directly on the same neurones; however, they appeared to be approximately equipotent.

#### *Effects of bicuculline on the actions of baclofen*

Applications of the GABA<sub>A</sub> receptor antagonist bicuculline that completely blocked f-i.p.s.p.s and responses to ionophoretically applied GABA had no effect on

baclofen's depressions of e.p.s.p.s (Fig. 15*A*) or on baclofen-induced hyperpolarizations and reductions of  $R_N$  (Fig. 15*B*). Bicuculline was applied at concentrations of 10–100  $\mu\text{M}$ . Applications of 1  $\mu\text{M}$ -bicuculline are sufficient to markedly reduce f.i.p.s.p.s or the responses of rat neocortical neurones to ionophoretically applied GABA or the GABA<sub>A</sub> agonist muscimol.

Although both compounds blocked f-i.p.s.p.s, the effects of baclofen and bicuculline were clearly different. On each neurone tested ( $n = 7$ ), baclofen decreased synaptic excitability, whereas bicuculline increased it (Fig. 16*A*). Baclofen increased the stimulation threshold for the bursts of action potentials that were recorded in the presence of bicuculline (Fig. 16*B*).

#### *Effects of baclofen on somatic calcium spikes*

Intracellular injections of caesium ions or superfusion with medium that contained 10 mM-TEA prolonged the duration of directly evoked action potentials 15–100-fold. The duration of such action potentials is reduced reversibly by manganese or cobalt ions, indicating that the increased duration is due to the inward movement of calcium ions when spike repolarization is inhibited. Applications of high concentrations of baclofen (50–200  $\mu\text{M}$ ) did not alter the duration of action potentials recorded in the presence of caesium or TEA in any of the neurones tested ( $n = 4$ , Fig. 17).

### DISCUSSION

#### *Effects of baclofen on stimulation-evoked post-synaptic potentials*

The depression by baclofen of e.p.s.p.s is consistent with reports from investigations in other regions of the mammalian C.N.S. (Pierau & Zimmermann, 1973; Fox *et al.* 1978; Blaxter & Carlen, 1985; Haas, Greene & Olpe, 1985; Inoue, Matsuo & Ogata, 1985*b*). As mentioned, the f-i.p.s.p.s we record in rat neocortical neurones are mediated by GABA<sub>A</sub> receptors (Sutor & Zieglgänsberger, 1984). Baclofen also reduces GABA<sub>A</sub>-receptor-mediated i.p.s.p.s evoked in neurones in the hippocampus and in the olfactory cortex (Misgeld *et al.* 1982; Scholfield, 1983; Blaxter & Carlen, 1985; Inoue *et al.* 1985*b*). The l-i.p.s.p.s we record resemble closely the long-duration (slow) i.p.s.p.s that are recorded from hippocampal pyramidal cells. Baclofen reduces the amplitude of slow i.p.s.p.s in CA1 and CA3 pyramidal cells (Blaxter & Carlen, 1985; Inoue *et al.* 1985*b*).

The principal effect of baclofen on rat neocortical neurones was to reduce synaptic excitability, although baclofen reduced both f-i.p.s.p.s and l-i.p.s.p.s. Baclofen also increased action potential stimulation thresholds in previous intracellular studies (Pierau & Zimmermann, 1973; Fox *et al.* 1978; Haas *et al.* 1985), although some investigators report that baclofen both increased and decreased action potential thresholds, depending on the cell population investigated and the concentration of baclofen applied (Misgeld *et al.* 1982; Inoue *et al.* 1985*b*).

The consistency of the increases in action potential stimulation threshold that we observed is in part explained by the characteristic sequence in which post-synaptic potentials are recruited in rat neocortical neurones with the stimulation we employed. Post-synaptic potential sequences evoked with subthreshold stimulation intensities are dominated by e.p.s.p.s, and baclofen's action to depress e.p.s.p.s is therefore of more consequence for these potential sequences than is its action to depress i.p.s.p.s.

In addition, baclofen decreased the direct excitability of most neurones tested and this effect also contributes to the action of baclofen to increase the stimulation threshold of the synaptically evoked action potential.

#### *Effects of baclofen on $E_m$ and $R_N$*

The mean reversal potential of baclofen-induced changes in  $E_m$  and the dependence of apparent  $E_{BAC}$  on the extracellular potassium concentration suggest these changes are secondary to an increase in the conductance of the post-synaptic membrane to potassium ions. Studies of the hyperpolarizing action of baclofen on hippocampal neurones (Newberry & Nicoll, 1984, 1985; Gähwiler & Brown, 1985; Inoue *et al.* 1985*a*) and on neurones in the dorsolateral septal nucleus (Stevens, Gallagher & Shinnick-Gallagher, 1985) have shown convincingly that baclofen directly increases the potassium conductance of these cells. The magnitude of the hyperpolarizations produced by baclofen in our experiments was typically only 2–6 mV. Larger amplitude hyperpolarizations in response to baclofen have been reported for other C.N.S. neurones (Misgeld *et al.* 1982; Brady & Swann, 1984; Pinnock, 1984; Haas *et al.* 1985; Inoue *et al.* 1985*a*; Newberry & Nicoll, 1985). The magnitudes of the decreases in apparent input resistance we observed were, however, very similar to those produced by baclofen in other C.N.S. neurones (Newberry & Nicoll, 1984; Blaxter & Carlen, 1985; Haas *et al.* 1985; Inoue *et al.* 1985*a*). The smaller amplitude hyperpolarizations we observed are therefore probably accounted for by the higher resting  $E_m$  values of the neurones we recorded.

In cultured hippocampal neurones, baclofen-induced currents exhibit inward rectification (Gähwiler & Brown, 1985). We recorded baclofen-induced outward currents at holding potentials of –55 to –65 mV, but we did not examine their voltage sensitivity. Inoue *et al.* (1985*a*) also concluded that baclofen-induced conductance changes exhibit inward rectification from their comparisons of control current–voltage relations (obtained during current-clamp recordings) to those obtained in the presence of baclofen. Our similar comparisons of current–voltage relations did not, in most cases, suggest that the conductance increases produced by baclofen were voltage dependent; nor did our estimations of  $R_N$  from single hyperpolarizing current steps applied at several different  $E_m$  values provide any consistent evidence for such voltage dependence. The baclofen-induced decreases in  $R_N$  were commonly quite modest, however, and the interpretation of such current-clamp measurements is complicated by the rectifying behaviour of the neurones themselves. As such, we believe that these results do not exclude a voltage dependence of the baclofen-induced conductance increases we observed. In all major respects, the conductance increases produced by baclofen in rat neocortical neurones are similar in character to those produced by baclofen in other central neurones. This action of baclofen results in decreases in direct excitability.

#### *Pharmacology of baclofen actions*

Our results indicate that the  $EC_{50}$  value for each action of baclofen observed is approximately 1  $\mu M$ . This is similar to estimated  $EC_{50}$  values for baclofen actions observed in other electrophysiologic studies (Ault & Nadler, 1982, 1983; Collins,



Anson & Kelly, 1982; Inoue *et al.* 1985*a*; Newberry & Nicoll, 1985). Depressions of e.p.s.p.s and i.p.s.p.s and hyperpolarizations and decreases in direct excitability became evident at concentrations of 10–100 nM, concentrations of baclofen that are approximately equal to those obtained in the cerebrospinal fluid after systemic administration of therapeutic doses in man (Knutsson, Lindblom & Martensson, 1974; Swahn, Beving & Sedvall, 1979).

Each action of baclofen was more than 100-fold stereoselective. Quantitatively similar estimations have been made for the stereoselectivity of baclofen actions observed in other electrophysiologic studies (Ault & Nadler, 1982, 1983; Brady & Swann, 1984; Haas *et al.* 1985; Inoue *et al.* 1985*a*; Newberry & Nicoll, 1985). Given the extent of this stereoselectivity, L-baclofen should be approximately twice as potent as DL-baclofen. The range of L-baclofen concentrations we applied and the number of neurones we tested were too small, however, to allow us to accurately discriminate a difference in potency of this magnitude. The results of further experiments do indicate, however, that as expected L-baclofen is slightly more potent than DL-baclofen on neocortical neurones (Howe & Zieglgänsberger, 1986).

In agreement with previous reports, baclofen-induced hyperpolarizations and decreases in  $R_N$  were insensitive to blockade by concentrations of the GABA<sub>A</sub> receptor antagonist bicuculline between 10 and 100  $\mu$ M (Brady & Swann, 1984; Newberry & Nicoll, 1984, 1985; Pinnock, 1984; but see Inoue *et al.* 1985*b*). Baclofen-induced outward currents are also not reduced by GABA<sub>A</sub> receptor blockade (Gähwiler & Brown, 1985).

It was concluded on the basis of extracellular recordings that baclofen's depressions of excitatory synaptic transmission are not blocked by GABA<sub>A</sub> receptor antagonists (Ault & Nadler, 1982; Cain & Simmonds, 1982; Collins *et al.* 1982; Inoue *et al.* 1985*b*). Our intracellular results which demonstrate that baclofen's depressions of e.p.s.p.s are resistant to antagonism by bicuculline provide direct support for this.

Although baclofen blocked i.p.s.p.s, the actions of baclofen and bicuculline on rat neocortical neurones were not synergistic, as they were reported to be on hippocampal granule cells (Misgeld *et al.* 1982). Thus, bicuculline consistently and significantly decreases the stimulation thresholds of action potentials in rat neocortical neurones, whereas baclofen consistently increased this threshold. In addition, baclofen increased, rather than decreased, the threshold stimulation intensity required to produce action potential bursts evoked in the presence of bicuculline (cf. Ault & Nadler, 1983; Brady & Swann, 1984). The concentrations of bicuculline that we used, however, produced apparently complete blockades of f-i.p.s.p.s and any further depression of these i.p.s.p.s by baclofen was probably precluded.

Although the resistance of the effects of baclofen to antagonism by bicuculline indicates that the effects of this GABA derivative are not secondary to GABA<sub>A</sub> receptor activation, we have no evidence that the actions of baclofen we observed are mediated by GABA<sub>B</sub> receptors. Thus, in the presence of bicuculline, we have never seen baclofen-like hyperpolarizations in response to ionophoretic applications of GABA. This is in contrast to results obtained in the hippocampus where GABA produces hyperpolarizations similar to baclofen when GABA<sub>A</sub> receptors are blocked with bicuculline (Newberry & Nicoll, 1985). These bicuculline-resistant hyperpolar-

izations are observed, however, when GABA is applied to the dendritic field of hippocampal neurones. In our experiments, GABA applications were made from ionophoretic electrodes that were placed as close to the recording electrode as possible, and therefore we have presumably applied GABA only near the soma of neocortical neurones.

*The relation between hyperpolarizations and conductance increases produced by baclofen and baclofen's depressions of stimulation-evoked post-synaptic potentials*

The following experimental findings indicate that, although the action of baclofen to increase post-synaptic potassium conductance certainly contributes to baclofen's depression of stimulation-evoked post-synaptic potentials, this action alone cannot account for the depressions of these potentials that were produced by baclofen. First, the effects could be dissociated. For several neurones, post-synaptic potentials were markedly reduced by baclofen, but changes in  $R_N$  were not detected. Conversely, in a few neurones baclofen produced hyperpolarizations and typical decreases in  $R_N$ , but the reductions of post-synaptic potentials were considerably less than those commonly observed. Secondly, whereas for the majority of neurones baclofen both decreased post-synaptic potentials and produced hyperpolarizations and detectable decreases in  $R_N$ , baclofen applications that produced 70–100% depressions of post-synaptic potentials, typically produced only 20–30% decreases in  $R_N$ . Thirdly, in virtually every neurone tested, baclofen's depression of post-synaptic potentials persisted at times when baclofen-induced decreases in  $R_N$  were no longer detectable. Post-synaptic potentials were often still significantly reduced several minutes after  $E_m$  and  $R_N$  had returned to control values. All of these results suggest that, in addition to reducing the amplitude of synaptically evoked e.p.s.p.s and i.p.s.p.s by decreasing the resistance of the membrane over which the currents that generate those post-synaptic potentials flow, baclofen must also reduce the amplitude of stimulation-evoked post-synaptic currents (e.p.s.c.s and i.p.s.c.s).

For both f- and l-i.p.s.p.s, this is verified by our findings that the conductance increases associated with each type of i.p.s.p.s were decreased by baclofen. Baclofen's depressions of i.p.s.p.s were independent of  $E_m$  and were not accompanied by changes in the reversal potential of either f-i.p.s.p.s or l-i.p.s.p.s. Thus baclofen does not reduce the driving force for the generation of either type of i.p.s.p., but reduces the magnitude of the conductance increases that precede them.

Although we did not perform  $E_{e.p.s.p.}$  determinations, baclofen's reductions of e.p.s.p. amplitudes were independent of  $E_m$  over the range of values  $\pm 25$  mV from resting  $E_m$ . Thus, it is suggested that baclofen does not alter the driving force for e.p.s.c.s, but reduces the conductance increases that lead to e.p.s.p. generation. We have not attempted to measure conductance increases during e.p.s.p.s, however, nor to record e.p.s.c.s under conditions of somatic voltage clamp. As such, the suggestion that baclofen must reduce e.p.s.c.s rests primarily on the above-stated results which indicate that the decreases of  $R_N$  that were measured during baclofen were not of sufficient magnitude or duration to account for baclofen's reductions of e.p.s.p. amplitudes.

This conclusion is only valid on the condition that we detected close to the full extent of the conductance increases produced by baclofen. This condition would not

be met if baclofen increases membrane conductance selectively at regions of the neurone that are electronically remote from the site of the recording electrode. If, in addition, e.p.s.p.s are generated at these same electrotonically remote regions, then it is conceivable that baclofen could selectively shunt e.p.s.p.s.

That typical decreases in  $R_N$  were measured during baclofen applications that produced quite modest reductions of post-synaptic potentials suggests this is not the case, but this was only observed in a few neurones. The large disparities, however, between the magnitude and duration of baclofen's reductions of e.p.s.p.s and its reductions of depolarizations produced by L-glutamate, L-aspartate or NMDA, also argue against such a hypothetical situation. Like stimulation-evoked e.p.s.p.s and unlike stimulation-evoked f-i.p.s.p.s or responses to ionophoretically applied GABA, depolarizations produced by these excitatory amino acids are influenced significantly by the non-linearities in the current-voltage relations of rat neocortical neurones. Depolarizations produced by these excitatory amino acids are often accompanied by apparent increases in  $R_N$  as a result of the inward rectification displayed by these neurones at  $E_m$  values negative to  $-60$  mV. Even when rectification is accounted for, the decreases in resistance during large (20–30 mV) subthreshold depolarizations produced by these substances are small.

These results suggest that depolarizations produced by these excitatory amino acids are generated at sites that are electrotonically remote from the recording electrode, and yet baclofen's effects on these depolarizations were quantitatively similar in magnitude and duration to its effects on depolarizations produced by direct intracellular current injections. It therefore seems unlikely to us that baclofen depresses e.p.s.p.s by selectively increasing membrane conductance at their electrotonically remote sites of generation. Our results do not directly exclude this possibility however.

#### *Mechanism of baclofen's reduction of stimulation-evoked post-synaptic conductances*

On the basis either of direct measurements during i.p.s.p.s, or from the indirect evidence just described for e.p.s.p.s, our results indicate that baclofen reduces the magnitude of the conductance increases that precede each post-synaptic potential. Because baclofen did not reduce either currents or conductance increases evoked by the direct ionophoretic application of GABA, a post-synaptic blockade by baclofen of the f-i.p.s.p. conductance is extremely unlikely. Scholfield (1983) also found no action of baclofen on conductance increases produced by the direct application of GABA or the GABA<sub>A</sub> agonist muscimol. Experiments in our laboratory with excitatory amino acid antagonists indicate that excitatory amino acids contribute significantly to the generation of e.p.s.p.s in our preparation (unpublished results). As such, our findings that baclofen did not significantly reduce depolarizations produced by L-glutamate, L-aspartate, or NMDA and that it did not reduce L-glutamate-evoked inward current suggest that baclofen does not act post-synaptically to reduce the conductance increases that generate e.p.s.p.s. Our results therefore suggest that baclofen reduces the available amount of the synaptically released transmitters that generate the f-i.p.s.p.s and e.p.s.p.s we record from rat neocortical neurones.

Indeed, baclofen has been shown to decrease the evoked release of several

neurotransmitters (Bowery *et al.* 1980; Olpe *et al.* 1982; Schlicker, Classen & Göthert, 1984), and importantly to reduce the release of excitatory amino acids in preparations of the mammalian neocortex (Potashner, 1979; Johnston, Hailstone & Freeman, 1980). Baclofen does not, however, reduce the release of GABA evoked by potassium depolarizations or electrical field stimulation from slices of the rat or guinea-pig neocortex (Potashner, 1979; Johnston *et al.* 1980).

In the olfactory cortex slice, Collins *et al.* (1982) also found that baclofen did not reduce potassium-evoked GABA release. Baclofen did, however, significantly reduce GABA release evoked by electrical stimulation of the lateral olfactory tract. They proposed that baclofen's reductions of this type of evoked GABA release, and also baclofen's apparent reductions of GABAergic post-synaptic inhibition, were secondary to its direct action to reduce excitatory amino acid neurotransmitter release and consequently the excitatory drive to GABA-releasing interneurons (see also Scholfield, 1983). A similar dependence of the activity of the GABAergic neurones that generate f.i.p.s.p.s in the rat neocortex on stimulation-evoked excitatory synaptic transmission would both explain our findings and be consistent with baclofen's lack of effect on directly evoked GABA release.

As mentioned, l-i.p.s.p.s are very similar to the so-called slow i.p.s.p.s evoked in hippocampal CA1 neurones. Because of the similarities between slow i.p.s.p.s and hyperpolarizations produced by baclofen, it was proposed that both may be secondary to activation of GABA<sub>B</sub> receptors (Newberry & Nicoll, 1984, 1985). If baclofen and the endogenous transmitter that generates l-i.p.s.p.s act on the same population of post-synaptic receptors or increase the same post-synaptic potassium conductance, then baclofen and the endogenous transmitter may compete for these receptors, or the conductance may already be fully activated in the presence of baclofen, and baclofen's reductions of l-i.p.s.p.s might at least in part be the result of its post-synaptic action. At present, this possibility cannot be tested directly because of the lack of established antagonists of either baclofen-induced hyperpolarizations or of l-i.p.s.p.s.

Dunlap (1981) showed that baclofen reduces the duration of the calcium component of action potentials recorded from dorsal root ganglion neurones in culture. Baclofen also reduces the duration of the calcium component of action potentials recorded from neurones in the myenteric plexus (Cherubini & North, 1984), and several authors have proposed that baclofen reduces neurotransmitter release by reducing inward calcium currents in presynaptic terminals. Although the results of Shapovalov & Shiriaev (1982) in the frog motoneurone are consistent with such a mechanism, Barry (1984) concluded that baclofen's presynaptic depression of excitatory transmission at the crayfish neuromuscular junction was unlikely to be due to a reduction of presynaptic calcium influx. Consistent reductions in the presynaptic component of stimulation-evoked calcium entry in the rat hippocampus were only observed at a baclofen concentration of 50  $\mu$ M (Heinemann, Hamon & Konnerth, 1984).

Baclofen had no significant effect, even at very high concentrations, on the duration of the calcium component of action potentials we recorded from rat neocortical neurones. Gähwiler & Brown (1985) found that baclofen had no effect on inward calcium currents recorded from cultured hippocampal neurones under

voltage-clamp conditions. Although neither our results nor those of Gähwiler and Brown argue against an effect of baclofen on calcium currents that occur in presynaptic terminals, they do indicate that baclofen's reduction of somatic calcium currents in dorsal root ganglion or myenteric plexus neurones do not necessarily extrapolate to C.N.S. neurones.

Davidoff & Sears (1974) proposed that baclofen's depressions of synaptic transmission might result from baclofen's action to hyperpolarize presynaptic terminals and consequently to reduce their excitability. Direct experimental evidence indicates, however, that baclofen does not decrease the excitability of presynaptic terminals to an extent sufficient to decrease the probability of action potential invasion of the terminal region (Fox *et al.* 1978; Capek & Esplin, 1982; Shapovalov & Shiriaev, 1982). Gähwiler & Brown (1985) noted, however, that if baclofen increased the potassium conductance of presynaptic terminals this might indirectly reduce presynaptic calcium influx by reducing the duration of the action potential. In our experiments, baclofen's depression of post-synaptic potentials usually outlasted the increases in post-synaptic conductance that were produced by baclofen by several minutes. Therefore, if baclofen increases the potassium conductance of presynaptic terminals in the rat neocortex, these conductance increases have different kinetics than those we have measured.

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